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(57) Abstract

Individuals having tumors are treated with pharmaceutical compositions comprising expression vectors, preferably adenovirus or retroviruses, comprising nucleic acid molecules encoding soluble IGF-1R. Such vectors express soluble IGF-1R in tumor cells resulting in reversal of the transformed phenotype, induction of apoptosis, and inhibition of tumorigenesis.

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INDUCING RESISTANCE TO TUMOR GROWTH WITH SOLUBLE IGF-1 RECEPTOR

REFERENCE TO GOVERNMENT GRANTS

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CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims priority under 35 U.S.C. § 119(e) to provisional patent application Serial No. 60/006,699, filed November 14, 1996, hereby incorporated in its entirety by reference.

FIELD OF THE INVENTION

The present invention relates to expression vectors that code for soluble type I insulin-like growth factor receptor (IGF-1R) which, when transfected into tumor cells, reverses the transformed phenotype, induces apoptosis, and inhibits tumorigenesis in syngeneic animals.

BACKGROUND OF THE INVENTION

It is well established that growth factors play a crucial role in the establishment and maintenance of the transformed phenotype. Evidence is rapidly accumulating that growth factor receptors also play a crucial role in the establishment and maintenance of transformed phenotypes.

The IGF-1R belongs to the family of tyrosine kinase growth factor receptors (Ullrich, *et al.*, *Cell*, **1990**, *61*, 203), and is 70% homologous to the insulin growth factor I receptor

(Ullrich, *et al.*, *EMBO J.*, **1986**, *5*, 503). The IGF-1R activated by its ligands (IGF-1, IGF-2 and insulin at supraphysiological concentrations) has been known to be mitogenic in cell cultures. However, in growth-regulated cells, like 3T3 cells and human diploid fibroblasts, IGF-1, by itself, cannot sustain growth of cells in serum-free medium (SFM), but requires the cooperation of other growth factors, for instance PDGF and/or EGF, which, by themselves, also fail to induce a mitogenic response. Scher, *et al.*, *Biochem. Biophys. Acta*, **1979**, *560*, 217; and Stiles, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1979**, *76*, 1279.

Recently, the importance of the IGF-1R in cell growth has been confirmed *in vivo* by the finding that mouse embryos with a targeted disruption of the IGF-1R and IGF-2 genes have a size at birth that is only 30% the size of wild type littermates. Liu, *et al.*, *Cell*, **1993**, *75*, 59; and Baker, *et al.*, *Cell*, **1993**, *73*, 73. 3T3-like cells derived from these mouse embryos devoid of IGF-1Rs (R^- cells) do not grow at all in SFM supplemented by a variety of growth factors, which can sustain the growth of cells derived from wild type littermate embryos (W cells) and other 3T3-cells. Sell, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 3604. R^- cells grow in 10% FBS at a rate that is roughly 40% the rate of W cells, with all phases of the cell cycle being equally elongated. Sell, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 3604. R^- cells are also refractory to transformation by SV40 large T antigen, by an activated *ras* or a combination of both (Sell, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 11217; and Sell, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 3604), or by overexpressed growth factor receptors, such as the EGF receptor (Coppola, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 4588), the PDGF β receptor (DeAngelis, *et al.*, *J. Cell. Physiol.*, **1995**, *164*, 214) and the insulin receptor (Miura, *et al.*, *Cancer Res.*, **1995**, *55*, 663), all conditions that readily transform cells from wild type littermate embryos or other 3T3-like cells with a physiological number of IGF-1Rs. Conversely, overexpression and/or constitutive activation of IGF-1R in a variety of cell types leads to ligand-dependent

growth in SFM and to the establishment of a transformed phenotype. Kaleko, *et al.*, *Mol. Cell. Biol.*, **1990**, *10*, 464; McCubrey, *et al.*, *Blood*, **1991**, *78*, 921; Pietrzkowski, *et al.*, *Mol. Cell. Biol.*, **1992**, *12*, 3883; Liu, *et al.*, *Cell*, **1993**, *75*, 59; Sell, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 3604; Coppola, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 4588; and Surmacz, *et al.*, *Exp. Cell Res.*, **1995**, *218*, 370.

The importance of the IGF-1 receptor in the control of cell proliferation is also supported by the observation that many cell types in culture are stimulated to grow by IGF-I (Goldring, *et al.*, *Crit. Rev. Eukaryot. Gene Expr.*, **1991**, *1*, 301; and Baserga, *et al.*, *Crit. Rev. Eukaryot. Gene Expr.*, **1993**, *3*, 47), and these cell types include human diploid fibroblasts, epithelial cells, smooth muscle cells, T lymphocytes, myeloid cells, chondrocytes, osteoblasts as well as the stem cells of the bone marrow. Using antisense expression vectors or antisense oligonucleotides to the IGF-1 receptor RNA, it has been shown that interference with IGF-1 receptor leads to inhibition of cell growth. The antisense strategy was successful in inhibiting cellular proliferation in several normal cell types and in human tumor cell lines. Baserga, *Cell*, **1994**, *79*, 927. Growth can also be inhibited using peptide analogues of IGF-1 (Pietrzkowski, *et al.*, *Cell Growth & Diff.*, **1992**, *3*, 199; and Pietrzkowski, *et al.*, *Mol. Cell. Biol.*, **1992**, *12*, 3883), or a vector expressing an antisense RNA to the IGF-1 RNA (Trojan, *et al.*, *Science*, **1993**, *259*, 94). The IGF autocrine or paracrine loop is also involved in the growth promoting effect of other growth factors, hormones (for instance, growth hormone and estrogens), and oncogenes like SV40 T antigen and *c-myb*, and in tumor suppression, as in the case of WT1 (Baserga, *Cell*, **1994**, *79*, 927).

The important role of IGF-1R in the establishment and maintenance of the transformed phenotype is supported by other findings. Antisense oligonucleotides or antisense expression plasmids against either IGF-2 (Christophori, *et al.*, *Nature*, **1994**, *369*, 414; and Rogler, *et al.*, *J. Biol. Chem.*, **1994**, *269*,

13779), IGF-1 (Trojan, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 4874; and Trojan, *et al.*, *Science*, **1993**, *259*, 94) or the IGF-1R (Sell, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 11217; Baserga, *Cell*, **1994**, *79*, 927; Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218; Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848; and Shapiro, *et al.*, *J. Clin. Invest.*, **1994**, *94*, 1235), antibodies to the IGF-1R (Arteaga, *et al.*, *Breast Canc. Res. Treatm.*, **1992**, *22*, 101; and Kalebic, *et al.*, *Cancer Res.*, **1994**, *54*, 5531), and dominant negative mutants of the IGF-1R (Prager, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 2181; and Li, *et al.*, *J. Biol. Chem.*, **1994**, *269*, 32558), can all reverse the transformed phenotype, inhibit tumorigenesis, and induce loss of the metastatic phenotype (Long, *et al.*, *Cancer Res.*, **1995**, *54*, 1006). An overexpressed IGF-1R has been found to protect tumor cells *in vitro* from etoposide-induced apoptosis (Sell, *et al.*, *Cancer Res.*, **1995**, *55*, 303) and, even more dramatically, that a decrease in IGF-1R levels below wild type levels caused massive apoptosis of tumor cells in vivo (Resnicoff, *et al.*, *Cancer Res.*, **1995**, *55*, 2463).

Expression of an antisense RNA to the IGF-1R RNA in C6 rat glioblastoma cells not only abrogates tumorigenesis in syngeneic rats, but also causes complete regression of established wild type tumors. Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218; and Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848. Cells expressing an antisense RNA to the IGF-1R RNA or cells pre-incubated with antisense oligonucleotides to the IGF-1R RNA completely lose their tumorigenicity when injected in either syngeneic or nude mice. Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218; and Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848. The injected cells were suspected of undergoing apoptosis or some form of cell death. Dying cells, however, are very difficult to demonstrate, because dying cells, especially *in vivo*, disappear very rapidly, and one is left with nothing to examine.

Tumors and other neoplastic tissues are known to undergo apoptosis spontaneously or in response to treatment. Examples include several types of leukemia, non-Hodgkin's

lymphoma, prostate tumor, pancreatic cancer, basal and squamous cell carcinoma, mammary tumor, breast cancer, and fat pad sarcoma. Several anticancer drugs have been shown to induce apoptosis in target cells. Buttyan, *et al.*, *Mol. Cell. Biol.*, 1989, *9*, 3473; Kaufmann, *Cancer Res.*, 1989, *49*, 5870; and Barry, *et al.*, *Biochem. Pharmacol.*, 1990, *40*, 2353. Certain mildly adverse conditions can result in the injured cell dying by programmed cell death, including hyperthermia, hypothermia, ischemia, and exposure to irradiation, toxins, and chemicals. It should be noted that many of these treatments will also result in necrosis at higher doses, suggesting that mild injury to a cell might induce a cell to commit suicide, perhaps to prevent the inheritance of a mutation, while exposure to severe conditions leads directly to cell death by necrosis.

Apoptosis refers to cell death, including, but not limited to, regression of primary and metastatic tumors. Apoptosis is a programmed cell death which is a widespread phenomenon that plays a crucial role in the myriad of physiological and pathological processes. There exists a homeostatic control of cell number thought to result from the dynamic balance between cell proliferation and cell death. In contrast, necrosis refers to an accidental cell death which is the cell's response to a variety of harmful conditions and toxic substances.

Apoptosis, morphologically distinct from necrosis, is a spontaneous form of cell death that occurs in many different tissues under various conditions. This type of cell death typically occurs in scattered cells and progresses so rapidly that it is difficult to observe.

The cell death process of apoptosis occurs in two stages. The cell undergoes nuclear and cytoplasmic condensation, eventually breaking into a number of membrane-bound fragments containing structurally intact apoptotic bodies, which are phagocytosed by neighboring cells and rapidly degraded. Apoptosis is observed in many different tissues, healthy and neoplastic, adult and embryonic. Death occurs spontaneously, or is induced by physiological or noxious

agents. Apoptosis is a basic physiological process that plays a major role in the regulation of cell populations.

The death process is difficult to observe due to the rapidity of the process and the reduced amount of inflammation. For these reasons, quantification of apoptosis is often difficult. A method of measuring the duration of the histologically visible stages of apoptosis (3 hours in normal rat liver) and present a formula by which to calculate the cell loss rate by apoptosis. Bursch, *et al.*, *Carcinogenesis*, **1990**, *11*, 847.

Nonetheless, testing agents such as growth factors and growth factor receptors for their ability to maintain or suppress transformed phenotypes remains difficult. In order to obtain an accurate account of the tumor suppressive ability, testing should be performed *in vivo*. Therapies such as direct injection or implantation of toxic treatments, tissue samples, and chemotherapy often jeopardizes the overall health of the patient. The present invention provides a method of inducing resistance to tumor growth with markedly reduced side effects to the patient.

SUMMARY OF THE INVENTION

The present invention relates to isolated soluble IGF-1R proteins.

The invention relates to isolated soluble IGF-1R proteins having amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof.

The invention relates to pharmaceutical compositions comprising isolated soluble IGF-1R in combination with a pharmaceutically acceptable carrier.

The invention relates to isolated nucleic acid molecules comprising nucleic acid sequences encoding soluble IGF-1R having an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof.

The invention relates to pharmaceutical compositions comprising nucleic acid molecules comprising nucleic acid sequences encoding soluble IGF-1R having an amino acid sequence

set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof, in combination with a pharmaceutically acceptable carrier.

The invention relates to isolated nucleic acid molecules consisting of sequences encoding soluble IGF-1R having an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof.

The invention relates to a recombinant expression vector comprising nucleic acid molecules having nucleic acid sequences encoding soluble IGF-1R having an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof.

The invention relates to a host cell comprising a recombinant expression vector comprising nucleic acid molecules comprising nucleic acid sequences encoding soluble IGF-1R having an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or fragments thereof.

The invention relates to methods of inducing apoptosis in tumor cells by contacting tumor cells with soluble IGF-1R.

The invention relates to methods of inducing apoptosis in tumor cells by transfecting tumor cells with nucleic acid molecules encoding soluble IGF-1R.

The invention relates to methods of treating individuals who have tumors comprising administering to such individuals a therapeutically effective amount of soluble IGF-1R.

The invention relates to methods of inducing apoptosis in tumor cells by introducing into the tumor cells a nucleic acid molecule comprising a nucleotide sequence encoding soluble IGF-1R operably linked to regulatory elements functional in the tumor cells wherein, upon introduction into the tumor cell, the nucleotide sequence that encodes soluble IGF-1R is expressed.

The invention relates to methods of treating individuals who have tumors comprising administering to such individuals a therapeutically effective amount of a nucleic acid molecules encoding soluble IGF-1R operably linked to regulatory sequences that function in tumor cells.

The invention relates to a method of inducing resistance to tumor growth in an individual comprising administering soluble IGF-1R for a therapeutically effective time.

The invention relates to a method of inducing resistance to tumor growth in an individual comprising providing a tumor cell culture supplemented with soluble IGF-1R in a diffusion chamber and inserting said chamber into an individual for a therapeutically effective time.

The invention relates to a method of inducing resistance to tumor growth in an individual comprising providing a tumor cell culture in a diffusion chamber and inserting said chamber into an individual for a therapeutically effective time, wherein the tumor cells are transfected with a gene construct comprising a nucleic acid molecule encoding soluble IGF-1R that has an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or a fragment thereof.

The invention relates to diffusion chambers comprising tumor cells with medium supplemented with soluble IGF-1R that has an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or a fragment thereof.

The invention relates to diffusion chambers comprising tumor cells with medium, wherein the tumor cells are transfected with a gene construct comprising nucleic acid molecule encoding soluble IGF-1R that has an amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2, or a fragment thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1G provide the amino acid and nucleotide sequence of IGF-1 receptor. Amino acids -30 to -1 represent the signal peptide sequence.

Figure 2 shows a schematic illustration of a diffusion chamber.

Figure 3 shows a graph of the growth of C6 cells and derived clones expressing soluble IGF-1R.

Figure 4 shows a graph of the effect of conditioned medium from cells expressing soluble IGF-1R on the growth of p6 cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and pharmaceutical compositions comprising soluble IGF-1R for inducing resistance to tumor cells. In preferred embodiments of the invention, individuals having tumors are treated with pharmaceutical compositions comprising gene therapeutic expression vectors, preferably adenovirus or retrovirus, comprising nucleic acid molecules encoding soluble IGF-1R. Such vectors express soluble IGF-1R in tumor cells resulting in reversal of the transformed phenotype, induction of apoptosis, and inhibition of tumorigenesis. In other embodiments of the invention, individuals having tumors are treated by implanting a diffusion chamber comprising tumor cells infected with expression vectors comprising nucleic acid molecules encoding soluble IGF-1R. Alternatively, the diffusion chamber comprises tumor cells cultured with soluble IGF-1R protein. In other embodiments of the invention, individuals having tumors are treated with pharmaceutical compositions comprising soluble IGF-1R protein.

Diseases in which cell elimination by induction of apoptosis include cancer, coronary restinosis after angioplasty, as well as autoimmune diseases. Tumors treatable with the methods of the present invention include and are not limited to melanoma, prostate, ovary, mammary, lungs, and smooth muscle tumors; as well as cells from glioblastoma, bone marrow stem cells, hematopoietic cells, osteoblasts, epithelial cells, fibroblasts. Those having ordinary skill in the art can readily identify individuals who are suspected of suffering from such diseases, conditions and disorders using standard diagnostic techniques.

As used herein, the term "soluble IGF-1R" refers to secreted IGF-1R proteins. Such proteins comprise up to about 800 amino acids of the N-terminus of IGF-1R, such that the C-

terminus transmembrane domain is completely deleted or is present to the extent that the protein comprising a portion of the transmembrane domain is not able to be anchored in the cell membrane. Preferably, soluble IGF-1R comprises the N-terminal 486 amino acids without a signal peptide (amino acids 1 to 486 as set forth in Figures 1A-1G; SEQ ID NO:1), or comprising 516 amino acids with a signal peptide (amino acids -30 to 486 as set forth in Figures 1A-1G; SEQ ID NO:2). In addition, the term "soluble IGF-1R" refers to fragments from about 10 amino acids to about 485 amino acids, such as IGF-1R proteins comprising N-terminal and C-terminal truncations or internal deletions.

In a preferred embodiment of the invention, resistance to tumor cells is induced by treating individuals having tumors with pharmaceutical compositions comprising gene therapeutic expression vectors, preferably adenovirus or retrovirus, comprising nucleic acid molecules encoding soluble IGF-1R. Gene therapeutic expression vectors having nucleic acid molecules encoding soluble IGF-1R serve as gene therapies for individuals to induce apoptosis of tumor cells, and the induction of a host response that kills or inhibits the growth of surviving tumor cells. This method has the following advantages: 1) soluble IGF-1R delivered into tumor cells induces apoptosis; 2) because it is soluble, the infected or transfected cells will produce soluble IGF-1R which will also cause apoptosis of non-transfected cells; and 3) the apoptotic cells induce a host response.

Gene therapy involves the introduction and stable insertion of genetic material into cells. Genetic material can generally be introduced into cells by, for example, transfection or transduction. Nucleic acid molecules encoding soluble IGF-1R are delivered using any one of a variety of transfection or transduction techniques, such as, for example, gene therapeutic expression vectors, *i.e.*, recombinant viral expression vectors, or other suitable delivery means, so as to affect their introduction and expression in compatible host cells.

Transfection refers to introduction of new genetic material into cells by incorporation of added DNA. Transfection can occur by physical or chemical methods. Many transfection techniques are known to those of ordinary skill in the art and include, for example, calcium phosphate DNA co-precipitation, DEAE-dextran DNA transfection, electroporation, and cationic liposome-mediated transfection. In addition, other delivery components are also contemplated such as transferrin-mediated transfection, retrotransposons (*see* U.S. Serial No. 5,354,674, incorporated herein by reference), targeted transfection nanoparticles (*see* U.S. Serial No. 5,460,831, incorporated herein by reference) and other receptor-mediated means.

Transduction refers to the process of transferring nucleic acid into cells using a DNA or RNA virus. In general, suitable viral vectors include, but are not limited to, DNA viruses such as recombinant adenoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses and Semliki Forest viruses and recombinant vaccinia viruses or RNA viruses such as recombinant retroviruses. Other recombinant vectors include recombinant prokaryotes which can infect cells and express recombinant genes.

The invention is intended to include such other forms of expression vectors and other suitable delivery means which serve equivalent functions and which become known in the art subsequently hereto.

Cells can be treated *in vivo* or *ex vivo*. For *ex vivo* treatment, cells are isolated from an animal (preferably a human), transformed (*i.e.*, transduced or transfected *in vitro*) with an expression vector comprising nucleic acid molecules encoding soluble IGF-1R, and then administered to a recipient. Procedures for removing cells from animals are well known to those of ordinary skill in the art. In addition to cells, tissue or the whole or parts of organs may be removed, treated *ex vivo* and then returned to the patient. Thus, cells, tissue or organs may be cultured, bathed, perfused and the like under conditions for introducing the expresion vector encoding

soluble IGF-1R into the desired cells. An example of *ex vivo* gene therapy is set forth in U.S. Serial No. 5,399,346, incorporated herein by reference.

For *in vivo* treatment, cells of an animal, preferably a mammal and most preferably a human, are transformed *in vivo* with an expression vector comprising nucleic acid molecules encoding soluble IGF-1R. The *in vivo* treatment may involve systemic treatment with a vector such as intravenously, local internal treatment with a vector such as by perfusion, topical treatment with a vector and the like. When performing *in vivo* therapy, the preferred vectors are based on noncytopathic eukaryotic viruses in which nonessential or complementable genes have been replaced with the gene of interest. Such noncytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have recently been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (*i.e.*, capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, "Gene Transfer and Expression, a Laboratory Manual", W.H. Freeman Co., New York (1990) and Murry, e.d. "Methods in Molecular Biology", Vol. 7, Humana Press, Inc., Cliffton, New Jersey (1991).

In preferred embodiments of the present invention, DNA is delivered to competent host cells by means of an adenovirus or parvovirus. One skilled in the art would readily understand this technique of delivering DNA to a host cell by

such means. Although the invention preferably includes adenovirus and parvovirus, the invention is intended to include any virus which serves equivalent functions. Viral expression vectors and methods of preparation thereof, such as for example, adenovirus and parvovirus, to transfect or infect host cells are disclosed in U.S. Serial No. 5,354,678, U.S. Serial No. 5,173,414, U.S. Serial No. 5,139,941, and U.S. Serial No. 5,252,479, all incorporated herein by reference in their entirety. In addition, gene therapeutic expression vectors preferably comprise cell-specific promoters which provide for expression of the linked gene in a cell-specific manner. Thus, in the present invention, a promoter can be chosen which provides for expression of soluble IGF-1R only in the tumor cell to be treated. Standard techniques for the construction of such vectors are well known to those skilled in the art, and can be found in references such as, for example, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), which is incorporated herein by reference.

Alternatively, RNA is delivered to competent host cells by means of a retrovirus. One skilled in the art would readily understand this technique of delivering RNA to a host cell by such means. Any retrovirus which serves to express the protein encoded by the RNA is intended to be included in the present invention.

In preferred embodiments of the present invention, the gene therapeutic expression vector, such as, for example, adenovirus, comprises a nucleic acid molecule encoding soluble human IGF-1 receptor incorporated in such a manner as to allow expression of soluble IGF-1R. A preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as: heat and lipid solvent stability, high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series

of transductions. Recent reports indicate that the adeno-associated virus can also function in an extrachromosomal fashion. Recombinant genomes that are between 50% and 110% of wild-type adeno-associated virus size can be easily packaged. Thus, a vector such as d13-94 can accommodate an insertion of the size required to encode soluble IGF-1R.

In preferred embodiments, a gene therapeutic expression vector encoding soluble IGF-1R can be constructed by removing all endogenous coding sequences (bases 190-4034) from an infectious molecular clone of an adeno-associated virus (pAV1 from ATCC, Rockville, MD). The RSV long terminal repeat (LTR) driven soluble IGF-1R and the Neo gene under the control of the SV40 early promoter is then inserted into this virus.

In addition, Semliki Forest virus vectors are useful as transducing agents and include, but are not limited to, pSFV1 and pSFV3-*lacZ* (Gibco-BRL). These vectors contain a polylinker for insertion of foreign genes therein which is followed by a series of stop codons. The gene of choice is inserted into the polylinker region and viruses are generated using the *in vitro* packaging helper virus system also provided by Gibco-BRL. Following the directions of the manufacturer and the disclosure contained herein, it is a relatively simple matter for one of skill in the art to generate Semliki Forest virus vectors capable of expressing soluble IGF-1R proteins of the invention.

Preferably, soluble IGF-1R is expressed in a cell-specific manner from a tumor cell-specific promoter. The nucleic acid sequence for introduction into gene therapeutic expression vectors can be derived from, for example, the CVN-IGF-1R plasmid (Ullrich, *et al.*, *EMBO J.*, 1986, *5*, 503, incorporated herein in its entirety by reference), which contains the full length coding sequence cDNA of the human IGF-1 receptor (*See* Figures 1A-1G) under the control of the SV40 promoter. The CVN-IGF-1R plasmid may be manipulated by well known techniques to produce additional gene constructs which encode soluble IGF-1R proteins of varying length in amino acids. Such gene constructs may be used to prepare gene

therapeutic expression vectors encoding soluble IGF-1R proteins of varying length.

For example, by using a frame shift mutation strategy, Applicants produced IGF-1R cDNA that produces a full-length mRNA but a truncated receptor, which is, after cleavage of the signal peptide, 486 amino acids long (SEQ ID NO:1). Alternatively, the soluble receptor can additionally consist of the signal peptide (amino acids -30 to -1 of Figure 1A), which then provides a soluble IGF-1R protein that is 516 amino acids long (SEQ ID NO:2). In addition, it is contemplated that soluble IGF-1R may comprise up to about 800 amino acids, as long as the transmembrane region of IGF-1R is such that it no longer anchors the receptor in the cell membrane. Additional gene constructs can be prepared by similar techniques to produce soluble IGF-1R proteins having a selected number of amino acids. The truncated receptor is secreted into the medium and can be detected by, for example, immunoprecipitation with antibodies.

Additional gene constructs encoding species of soluble IGF-1R may be constructed as desired. For example, fragments of soluble IGF-1R may be produced which retain the ability to induce apoptosis. Such fragments can be, for example, C-terminal truncations, N-terminal truncations, and proteins comprising internal deletions. The fragments can be as long as 515 amino acids, for proteins comprising the signal peptide, and 485 amino acids for proteins not comprising the signal peptide, and as short as 10 amino acids at the N-terminus, C-terminus, or internal portion of the IGF-1R protein. Such species may be constructed by using techniques such as, for example, site-specific mutagenesis, and similar techniques which are well within the ability of the art skilled. Thus, the present invention also contemplates soluble IGF-1R proteins and gene constructs encoding proteins having portions of the complete IGF-1R sequence. Moreover, conservative amino acid substitutions may be made throughout the protein without significantly reducing apoptosis activity.

Pharmaceutical compositions of the invention comprise gene therapeutic expression vectors having nucleic acid molecules encoding soluble IGF-1R, such as those set forth above. Such nucleic acid molecules induce apoptosis and inhibit tumor growth in individuals who have tumors. Pharmaceutical compositions according to the invention comprise a pharmaceutically acceptable carrier in combination with nucleic acid molecules encoding soluble IGF-1R. Pharmaceutical formulations are well known and pharmaceutical compositions comprising nucleic acid molecules encoding soluble IGF-1R may be routinely formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference. The present invention relates to an injectable pharmaceutical composition that comprises a pharmaceutically acceptable carrier and nucleic acid molecule encoding soluble IGF-1R. Nucleic acid molecule encoding soluble IGF-1R is preferably sterile and combined with a sterile pharmaceutical carrier.

In some embodiments, for example, nucleic acid molecules encoding soluble IGF-1R can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (*e.g.*, sodium chloride, mannitol) and chemical stability (*e.g.*, buffers and preservatives). The formulation is sterilized by commonly used techniques.

An injectable composition may comprise nucleic acid molecules encoding soluble IGF-1R in a diluting agent such as, for example, sterile water, electrolytes/dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and polyethylene glycol. The injectable must be sterile and free of pyrogens.

Pharmaceutical compositions according to the invention include delivery components in combination with nucleic acid molecules that encode soluble IGF-1R which further comprise a pharmaceutically acceptable carriers or vehicles, such as, for example, saline. Any medium may be used which allows for successful delivery of the nucleic acid. One skilled in the art would readily comprehend the multitude of pharmaceutically acceptable media that may be used in the present invention.

The pharmaceutical compositions of the present invention may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. Pharmaceutical compositions may be administered parenterally, *i.e.*, intravenous, subcutaneous, intramuscular. Intravenous administration is the preferred route. Alternatively, tumor cells may be removed from an individual, and nucleic acid molecules encoding soluble IGF-1R protein introduced therein *in vitro* by techniques such as, for example, nake DNA transfection, microinjection, cell fusion, infection with virions, *etc.*

Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired.

In another embodiment of the present invention, individuals having tumors can be treated by implanting a diffusion chamber comprising medium and tumor cells infected or transfected with expression vectors comprising a nucleic acid molecule encoding soluble IGF-1R.

Resistance to tumor growth is induced by placing tumor cells supplemented with nucleic acid molecules encoding soluble IGF-1R in a diffusion chamber thereby producing a cell-containing chamber, inserting the cell-containing chamber into a mammal for a therapeutically effective time, thereby inducing resistance to tumor growth. Mammals subsequently

subcutaneously challenged with wild-type tumor cells are resistant to the tumor cells. In addition, regression of already established tumors is evidenced. The present invention is also directed to a method of inducing apoptosis. This application is related to U.S. application Serial No. 08/340,732, filed November 16, 1994, which is incorporated herein in its entirety by reference.

The tumor cells can be transfected with a nucleic acid molecule encoding a protein encoding soluble IGF-1R, such as the protein having the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, or a fragment thereof. The diffusion chamber containing the IGF-1R-infected cells is implanted for a therapeutically effective time. A therapeutically effective time is a time permitting death of the cells in said diffusion chamber and resistance of tumor growth in the mammal. The soluble IGF-1R produced by the tumor cells causes the death of the tumor cells in the chamber and elicits a host response such that the cell death has a growth inhibiting effect, *i.e.*, a resistant effect, on a tumor or tumors in the mammal in which the chamber is placed. Tumors which are treatable with the methods of the present invention may be primary or secondary, benign, malignant, metastatic, or micrometastatic tumors.

Therapeutically effective doses of nucleic acid molecules encoding soluble IGF-1R will be about that amount of nucleic acid alone; dosages will be set with regard to weight, and clinical condition of the patient. The proportional ratio of active ingredient to culture medium will naturally depend on the chemical nature, solubility, and stability of the compounds, as well as the dosage contemplated. The culture medium is also pharmaceutically acceptable.

Tumorous tissue may be placed in culture together with nucleic acid molecules encoding soluble IGF-1R. Tumorous tissue may be excised from the patient in which the diffusion chamber will be inserted, however, tumorous tissue from another source, and/or that which has been cultured *in vitro*, may also be used together with nucleic acid molecules encoding soluble IGF-1R. The tumor cells are cultured for a therapeutically

effective amount of time such that apoptosis of these cells is induced thereby causing resistance to tumor growth.

Before placing the cell-containing diffusion chamber into a mammal, cells may be gently dissociated with trypsin, incubated with nucleic acid molecules encoding soluble IGF-1R, and placed in the chamber. The chamber may then be implanted. Accordingly, any adverse treatments to the cells are performed *in vitro* thereby eliminating adversity to the host mammal. In addition, cells may be placed into a chamber and the chamber directly implanted into a mammal.

The present invention employs the use of a diffusion chamber, in which the cells are contained. Cells are impermeable to a filter fitted on the diffusion chamber; they cannot leave or enter the chamber. The filter on the diffusion chamber has pores in the size range of about 0.25 μm or smaller, preferably about 0.1 μm in diameter. Lange, *et al.*, *J. Immunol.*, **1994**, *153*, 205; and Lanza, *et al.*, *Transplantation*, **1994**, *57*, 1371, both incorporated herein by reference in their entirety. Accordingly, cell death or apoptosis, can be quantitatively determined. The use of a diffusion chamber can be extended to other cell lines, even non-syngeneic, and even from different species, because of the rapidity with which cell death occurs, about 24 hours, well before any immune reaction could be established. Indeed, 3 types of cells with an intact number of IGF-1Rs (human melanoma, rat rhabdomyosarcoma and murine p6 cells), double in number in 24 hours, regardless of whether they are syngeneic or not, while cells with decreased number of IGF-1Rs, die.

Diffusion chambers useful in the present invention include any chamber which does not allow passage of cells between the chamber and the mammal in which it is implanted, however, permits interchange and passage of factors between the chamber and the mammal. The chamber may allow for multiple and sequential sampling of the contents, without contamination and without sacrificing the mammal, therefore significantly reducing the number of implantation procedures performed on the mammal.

Referring to Figure 2, the diffusion chamber (1) may have a chamber barrel (3) having two ends, a first end (5) and a second end (7). The barrel may be comprised of one or more rings secured together by non-toxic means. The chamber is fitted at each end with a filter, a first filter (9) and a second filter (11). The filters are porous to factors such that the factors may pass between the chamber and the mammal. The filter pores size may be about 0.25 μm or smaller, preferably about 0.1 μm. The filters may be made of plastic, teflon, polyester, or any inert material which is strong, flexible and able to withstand chemical treatments. The filters may be secured in position with rubber gaskets which may also provide a tighter seal. On the barrel portion of the chamber, an opening (13) is provided which may be covered by a cap which is accessed from outside of the mammal's body once the chamber is implanted. The cap may be screw on type of self sealing rubber and fitted to the opening. Sampling of the chamber contents may thus be performed by accessing the opening by removing the cap on the outside of the mammal's body and inserting an ordinary needle and syringe. The chamber may be made of any substance, such as and not limited to plastic, teflon, lucite, titanium, or any inert material, which is non-toxic to, and well tolerated by, mammals. In addition, the chambers should be able to survive sterilization.

The chamber may be implanted in the following non-limiting ways: subcutaneously or intraperitoneally, for example. The chamber may be removed about 24 to about 30 hours after implantation. Alternatively, a refillable chamber may be employed such that the chamber may be re-used for treatments and emptied following treatments.

Tumor cells used in the diffusion chambers of the present invention include and are not limited to autografts, allografts, syngeneic, non-syngeneic and xenografts. Cells which may be cultured in a medium supplemented with nucleic acid molecules encoding soluble IGF-1R in a diffusion chamber include any type of cell which upon apoptosis induces resistance to tumor growth, including and not limited to tumor

cells. Preferably, tumor cells are placed in a diffusion chamber which is implanted in a mammal, wherein the tumor cells may preferably be the same type of tumor to which resistance is induced. However, an embodiment of the present invention includes tumors cultured in a diffusion chamber which are of a different type than the tumor to which resistance is granted. In addition, any type of cell which undergoes apoptosis and induces resistance to tumor growth is useful in the present invention.

In another embodiment of the present invention, individuals having tumors are treated by implanting a diffusion chamber comprising tumor cells in medium supplemented with soluble IGF-1R protein, or a fragment thereof, in a manner similar to that described above for nucleic acid molecules encoding soluble IGF-1R. The soluble IGF-1R which supplements the tumor cell culture in the diffusion chamber may be selected from any species of soluble IGF-1R, such as and not limited to, a protein having the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, or a fragment thereof.

The tumor cells can be treated with soluble IGF-1R, such as the protein having the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, or a fragment thereof. The diffusion chamber containing the IGF-1R-treated cells is implanted for a therapeutically effective time, as set forth above. Therapeutically effective doses of soluble IGF-1R will be about that amount of soluble IGF-1R alone; dosages will be set with regard to weight, and clinical condition of the patient. The proportional ratio of active ingredient to culture medium will naturally depend on the chemical nature, solubility, and stability of the compounds, as well as the dosage contemplated. The culture medium is also pharmaceutically acceptable.

In another embodiment of the present invention, individuals having tumors are treated with pharmaceutical compositions comprising soluble IGF-1R protein. Soluble IGF-1R preferably comprises the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2. Soluble IGF-1R can be administered as

a pharmaceutical composition, such as those described above for nucleic acid molecules encoding IGF-1R, to a mammal topically, intradermally, intravenously, intramuscularly, intraperitoneally, subcutaneously, and intraosseously.

The present invention provides substantially purified soluble IGF-1R which has the amino acid sequence set forth in SEQ ID NO:1, or SEQ ID NO:2 or fragments thereof. Soluble IGF-1R can be produced by recombinant DNA methods or synthesized by standard protein synthesis techniques.

Soluble IGF-1R proteins of the present invention can be prepared using recombinant DNA methods to produce a gene construct comprising a nucleic acid molecule encoding soluble IGF-1R protein, or portions thereof, including initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signals capable of directing expression in host cells. The regulatory elements of the gene constructs of the invention are capable of directing expression in mammalian cells, specifically human cells. The regulatory elements include a promoter and a polyadenylation signal. In addition, other elements, such as a Kozak region, may also be included in the gene construct.

The gene construct is inserted into an appropriate vector, *i.e.*, an expression plasmid, cosmid or YAC vector. Promoters and polyadenylation signals used must be functional within the host cells. In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cells into which the construct is administered. Moreover, codons may be selected which are most efficiently transcribed in the cell. One having ordinary skill in the art can produce DNA constructs which are functional in host cells.

Examples of promoters useful to practice the present invention include but are not limited to promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the Long Terminal Repeat (LTR) promoter, Maloney Virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr

Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human actin, human myosin, human hemoglobin, human muscle creatine and human metalothionein.

Examples of polyadenylation signals useful to practice the present invention include but are not limited to SV4 polyadenylation signals and LTR polyadenylation signals. In particular, the SV4 polyadenylation signal which is in pCEP4 plasmid (Invitrogen, San Diego, CA), referred to as the SV40 polyadenylation signal, can be used.

One having ordinary skill in the art can obtain a nucleic acid molecule that encodes soluble IGF-1R and insert it into an expression vector using standard techniques and readily available starting materials. The present invention relates to a recombinant expression vector that comprises a nucleic acid molecule having a nucleotide sequence that encodes soluble IGF-1R. As used herein, the term "recombinant expression vector" is meant to refer to a plasmid, phage, viral particle or other vector which, when introduced into an appropriate host cell, contains the necessary genetic elements to direct expression of the coding sequence that encodes the soluble IGF-1R of the invention. The coding sequence is operably linked to the necessary regulatory sequences. Expression vectors are well known and readily available. Examples of expression vectors include plasmids, phages, viral vectors and other nucleic acid molecules or nucleic acid molecule containing vehicles useful to transform or transfect host cells and facilitate expression of coding sequences. The recombinant expression vectors of the invention are useful for transforming or transfecting host cells to prepare recombinant expression systems for preparing soluble IGF-1R of the invention.

In some embodiments, for example, one having ordinary skill in the art can, using well known techniques, insert such DNA molecules into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for production of proteins in *E. coli*. The

commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may, for example, be used for production in *S. cerevisiae* strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, CA) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I (Invitrogen, San Diego, CA) may, for example, be used for production in mammalian cells such as CHO cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce soluble IGF-1R of the invention using routine techniques and readily available starting materials. *See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989) which is incorporated herein by reference. Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. *See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989). Commonly used eukaryotic systems include, but is not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, *e.g.* the baculovirus polyhedron promoter. As above, promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothionein promoter can be induced by the addition of heavy metal ions. The particulars for the construction of expression systems suitable for desired hosts are known to those in the art. Briefly, for recombinant production of the protein, the DNA encoding the polypeptide is

suitably ligated into the expression vector of choice. The DNA is operably linked to all regulatory elements which are necessary for expression of the DNA in the selected host. One having ordinary skill in the art can, using well known techniques, prepare expression vectors for recombinant production of the polypeptide.

The expression vector including the DNA that encodes soluble IGF-1R is used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign DNA takes place. The protein of the present invention thus produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art. One having ordinary skill in the art can, using well known techniques, isolate soluble IGF-1R that is produced using such expression systems. The methods of purifying soluble IGF-1R include using antibodies which specifically bind to soluble IGF-1R in immunoaffinity methodology.

Examples of genetic constructs include soluble IGF-1R operably linked to a promoter that is functional in the cell line into which the constructs are transfected. Examples of constitutive promoters include promoters from cytomegalovirus or SV40. Examples of inducible promoters include mouse mammary leukemia virus or metallothionein promoters. Those having ordinary skill in the art can readily produce genetic constructs useful for transfecting with cells with DNA that encodes soluble IGF-1R from readily available starting materials.

In addition to producing these proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce soluble IGF-1R of the invention. Such techniques are well known to those having ordinary skill in the art and are useful if derivatives which have substitutions not provided for in DNA-encoded protein production.

The present invention relates to host cells that comprise the recombinant expression vector that includes a nucleic acid molecule having a nucleotide sequence that encodes

soluble IGF-1R. Host cells for use in well known recombinant expression systems for production of proteins are well known and readily available. Examples of host cells include bacteria cells such as *E. coli*, yeast cells such as *S. cerevisiae*, insect cells such as *S. frugiperda*, non-human mammalian tissue culture cells chinese hamster ovary (CHO) cells and human tissue culture cells such as HeLa cells.

The present invention relates to a transgenic non-human mammal that comprises the recombinant expression vector that comprises a nucleic acid sequence that encodes soluble IGF-1R. Transgenic non-human mammals useful to produce recombinant proteins are well known as are the expression vectors necessary and the techniques for generating transgenic animals. Generally, the transgenic animal comprises a recombinant expression vector in which the nucleotide sequence that encodes soluble IGF-1R of the invention is operably linked to a mammary cell specific promoter whereby the coding sequence is only expressed in mammary cells and the recombinant protein so expressed is recovered from the animal's milk.

In some embodiments of the invention, transgenic non-human animals are generated. The transgenic animals according to the invention contain nucleotide sequences that encode soluble IGF-1R under the regulatory control of a mammary specific promoter. One having ordinary skill in the art using standard techniques, such as those taught in U.S. Patent No. 4,873,191 issued October 10, 1989 to Wagner and U.S. Patent No. 4,736,866 issued April 12, 1988 to Leder, both of which are incorporated herein by reference, can produce transgenic animals which produce soluble IGF-1R. Preferred animals are rodents, particularly rats and mice, and goats.

The present invention underscores the massive apoptosis *in vivo* induced by transfected or transduced soluble IGF-1R, which makes it a good candidate for inhibition of tumorigenesis and tumor regression in human patients. Treatment with soluble IGF-1R not only induces apoptosis of tumor cells, but also induces a host response that kills tumor cells not expressing soluble IGF-1R. Thus, delivery of soluble

IGF-1R into tumor cells would kill the cells that have taken up the vector carrying it and this would start a secondary response that kills the surviving tumor cells. This double-edged sword would make this approach very effective. It is effective in animals, where it induces apoptosis and elicits a host response, with little effect on normal cells. Because it is secreted, soluble IGF-1R will affect surrounding cells, and therefore increase its effectiveness. The application of soluble IGF-1R intends to cover all uses against various types of tumor cells, regardless of delivery route, whether they are primary tumors, residual tumors or metastases.

For purposes of the present invention, mammals include and are not limited to the Order Rodentia, such as mice; Order Logomorpha, such as rabbits; more particularly the Order Carnivora, including Felines (cats) and Canines (dogs); even more particularly the Order Artiodactyla, Bovines (cows) and Suines (pigs); and the Order Perissodactyla, including Equines (horses); and most particularly the Order Primates, Ceboids and Simoids (monkeys) and Anthropoids (humans and apes). The mammals of most preferred embodiments are humans.

The following examples are illustrative but are not meant to be limiting of the invention.

EXAMPLES

Example 1: Construction of Soluble IGF-1R Plasmid

A soluble IGF-1R protein truncated at amino acid residue 486 was prepared by the following method. Methods for the production and activity of soluble IGF-1R are also disclosed in D'Ambrosio, *et al.*, *Cancer Res.*, **1996**, *56*, 4013, incorporated in its entirety herein by reference. The CVN-IGF-1R plasmid (Ullrich, *et al.*, *EMBO J.*, **1986**, *5*, 503, incorporated herein in its entirety by reference), containing the full length coding sequence cDNA of the human IGF-1 receptor, under the control of the SV40 promoter, was digested with AgeI, which cuts at nucleotide 1574 (numbering according to Ullrich, *et al.*, *EMBO J.*, **1986**, *5*, 503). The overhangs were filled with Klenow and the plasmid was religated. This

procedure generates a frame shift mutation that results in the creation of an early stop codon 12 nucleotides downstream from the AgeI site. The mutation was confirmed by sequencing both strands (not shown). The wild type and mutant DNA sequences, and their translation, in the area corresponding to the mutation are shown below. The AgeI site is underlined. The mutation abrogates this restriction ite.

Wild type

... TGG CAC CGG TAC CGG CCC CCT GAC TAC...

... W H R Y R P P D Y...

Mutant

... TGG CAC CGG CCG GTA CCG GCC CCC TGA CTAC...

... W H R P V P A P *

WHR are amino acids 509-511, if the signal peptide is included (479-481 without the signal peptide). The soluble receptor is, therefore, 516 amino acids long (SEQ ID NO:2) or 486 (SEQ ID NO:1) without the signal peptide. The expression plasmid containing the nucleic acid sequence encoding soluble IGF-1R, and containing the neo-resistance gene, is designated pIGFIRsol. Additional soluble IGF-1R proteins truncated at other positions can be constructed using similar techniques.

Example 2: Preparation of pGEX Fusion Protein

Soluble IGF-1R can be expressed as a fusion protein. The pGEX-5x-3/IGFIRsol, a plasmid encoding the IGF-1R soluble protein, was prepared as follows. A PCR fragment corresponding to the soluble receptor (without the signal peptide) was created using mutagenic primers. The 5' primer GGATCCTAGAAATCTGCGGGCCAGGC, SEQ ID NO:3, contains an artificial BamHI site followed by the cDNA sequence, starting at nucleotide 135 and ending at nucleotide 153. The 3' reverse primer TCAGGGGGCCGGTACCGGCC, SEQ ID NO:4, contains two mismatches compared to the original cDNA sequence, resulting in a disruption of the AgeI restriction site. After sequencing on both strands, the PCR fragment was subcloned in the EcoRI site of the pCRII vector (InVitrogen), amplified and digested with BamHI and EcoRI.

pGEX-5x-3/IGFIRsol was constructed by digesting pGEX-5x-3 with BamHI-EcoRI and ligating the BamHI-EcoRI IGFIRsol fragment. Five colonies of BL21(DE3) transformed bacteria were selected, stimulated with 0.1 mM IPTG and checked for the GST fusion protein levels by SDS-PAGE and Coomassie blue staining. One colony expressing the highest level of GST fusion protein was chosen for large scale amplification. The GST fusion protein was then purified according to the protocol suggested by GST purification modules manufacturer (Pharmacia).

Example 3: Immunoprecipitation of Soluble IGF-1R

The GST/IGFIRsol protein was immunoprecipitated overnight with anti-GST polyclonal antibody (Santa Cruz Lab) and Protein-A Agarose anti-mouse Ig (Oncogene Science). Alternatively, soluble IGF-1R may be immunoprecipitated with antibodies to IGF-1R (α domain of the IGF-1R; cat# sc-712; Santa Cruz Lab). These antibodies may be stained with anti-rabbit IgG peroxidase conjugated antibody (Oncogene Science) and detected with an ECL system (Amersham).

Antibodies to soluble IGF-1R are preferably monoclonal antibodies, which are commercially available. The antibodies are preferably raised against soluble IGF-1R protein made in human cells. Immunoassays are well known and there design may be routinely undertaken by those having ordinary skill in the art. Those having ordinary skill in the art can produce monoclonal antibodies which specifically bind to soluble IGF-1R protein using standard techniques and readily available starting materials. The techniques for producing monoclonal antibodies are outlined in Harlow, E. and D. Lane, (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor NY, which is incorporated herein by reference, provide detailed guidance for the production of hybridomas and monoclonal antibodies which specifically bind to target proteins. It is within the scope of the present invention to include FAbs and $F(Ab)_2$s which specifically bind to soluble IGF-1R protein in place of antibodies.

Briefly, the soluble IGF-1R protein is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to the soluble IGF-1R protein, the hybridoma which produces them is cultured to produce a continuous supply of anti-soluble IGF-1R protein specific antibodies.

Example 4: Preparation of Tumor Cell Lines

The C6 rat glioblastoma cell line was used in several experiments. The C6 cell line is syngeneic in BD-IX rats (Charles River Breeders Laboratories, Boston, MA). This cell line has been described in detail by Trojan, *et al.*, *Science*, **1993**, *259*, 94; Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218; Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848; and Trojan, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 4874, the disclosures of which are hereby incorporated by reference in their entirety. Other cell lines used were a human melanoma cell line, FO-1, and a rat rhabdomyosarcoma cell line, BA 1112. Martin, *et al.*, *Eur. J. Cancer Clin. Oncol.*, **1983**, *19*, 791, incorporated herein by reference in its entirety. The cells were pre-incubated at 39°C for 24 hours, before inoculation in the diffusion chambers.

Cells were passaged in RPMI 1640 supplemented with 5% calf serum and 5% fetal bovine serum. $8x10^4$ cells were plated in 35 mm dishes in 10% serum; after 12 hours, the growth medium was removed and replaced with serum-free medium supplemented with 0.1% bovine serum albumin (fraction V) and 1.0 μM ferrous sulfate, with or without IGF-1 (10 ng/ml), as disclosed by Resnicoff, *et al.*, *Cancer Res.*, **1994**, *5*, 2218, incorporated herein by reference in its entirety.

Balb/c 3T3 are 3T3 cells, passaged for several years, and p6 cells are Balb/c 3T3 cells stably transfected with, and overexpressing a human IGF-1R cDNA. Pietrzkowski, *et al.*, *Cell Growth & Diff.*, **1992**, *3*, 199, incorporated herein by reference

in its entirety. (tsA)R$^-$ and (tsA)R$^+$ cells have been described by Sell, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1993, *90*, 11217. (tsa)R$^-$ cells have no IGF-1 receptors, while (tsa)R$^+$ overexpresses a human IGF-1R cDNA. Both (tsa)R$^-$ and (tsa)R$^+$ express SV40 T antigen.

To establish C6 cells expressing IGFIRsol, C6 cells were co-transfected with the IGFIRsol plasmid and pPDV6$^+$ plasmid, containing the puromycin resistance gene. DeAngelis, *et al.*, *J. Cell. Physiol.*, 1995, *164*, 214, incorporated herein in its entirety. Cells were selected in 2.5 mg/ml of puromycin; the resulting clones were switched to medium containing 1,400 mg/ml of G418 to keep them under strict selection (the pIGFIRsol plasmid contains the neo-resistance gene). Balb/IGFIRsol were obtained by stable transfection with the IGFIRsol plasmid and subsequent selection in 1,400 mg/ml of G418. R$^-$ cells were co-transfected with the pIGFIRsol plasmid and pdeltaSUpac (also containing the puromycin resistance gene), and the cells were selected in 2.5 μg/ml of puromycin.

Example 5: Diffusion Chamber

Diffusion chambers were constructed from 14 mm Lucite rings with 0.1 μm pore-sized hydrophilic Durapore membranes (Millipore, Bedford, MA). The diffusion chambers were sterilized with ethylene oxide prior to use. After the cells were pre-incubated for 24 hours according to the methods of Resnicoff, *et al.*, *Cancer Res.*, 1994, *54*, 2218, incorporated herein by reference in its entirety, and as set forth above, they were placed into the chambers, which were then inserted into the subcutaneous tissue of rats, under anesthesia with Halothane (inhalant).

Aliquots of $5x10^5$ cells were placed in diffusion chambers, that were then inserted in the subcutaneous tissue of syngeneic rats, and removed at various intervals thereafter. The number of cells in each chamber were counted, also the percentage of cells stained by trypan blue, and finally, the residual cells were plated in tissue culture dishes. Cell

death occurs so rapidly and because cells in a diffusion chamber are, at least in part, protected from an immune response. Lanza, *et al.*, *Transplantation*, **1994**, *57*, 1371. Cell recovery is the real measure of growth and survival, since viability of the recovered cells (as determined by trypan blue) was close to 100%.

Example 6: Growth Curves

Two different types of experiments were performed on C6 and C6/IGFIRsol cells. First, C6 and C6/IGFIRsol cells were seeded at the density of $3x10^4$ cells/35 mm dishes and switched to SFM or SFM + IGF-1 (20 ng/ml) after 24 hours. Second, C6 cells were seeded at the density of $8x10^4$ cells/35 mm dishes and switched after 24 hours to conditioned medium from C6 or C6/IGFIRsol cells.

Conditioned medium from R^-/IGFIRsol and Balb/IGFIRsol sells was tested on p6 cell growth. p6 cells were plated at a density of $3x10^4$ cells/35 mm dishes in DMEM containing 10% FBS. After 24 hours, the cells were washed with Hank's solution and growing medium was replaced with conditioned medium from different R^-/IGFIRsol or Balb/IGFIRsol clones and parental cell lines, alone or with IGF-1 (20 ng/ml). p6 in SFM or SFM + IGF-1 (20 ng/ml) were used as a control.

In every experiment, the growth response was determined after 96 hours of culture, by harvesting and counting the cells in a hemocytometer. All experiments were done in triplicate.

Example 7: Determination of Apoptosis

Briefly, a diffusion chamber was implanted into the subcutaneous tissue of rats or mice. The diffusion chamber, as disclosed in Abraham, *et al.*, *J. Parasitol.*, **1993**, *79*, 571, contains tumor cells supplemented with soluble IGF-1R, as a protein or expressed from an expression vector as described above. The tumor cells in the diffusion chamber behave essentially as cells injected into the subcutaneousl tissue of

animals. The diffusion chamber was removed after a period of time and the cells removed.

Cells were lysed in 50 μl of lysis buffer (10 mM EDTA, 50 mM Tris pH 8,0.5% sodium dodecyl sulfate, 0.5 mg/ml proteinase K). RNAse A (0.5 mg/ml) was added and lysates were incubated for 1 hour at 37°C. Two phenol extraction (equal volumes) were performed, followed by one chloroform extraction. DNA was precipitated with two volumes of ice-cold ethanol and incubated at -80°C for 1 hour. DNA was pelleted by centrifugation at 14,000 rpm for 10 minutes at 4°C. Pellets were air-dried for 30 minutes, resuspended in 50 μl of Tris-EDTA pH 8. DNA was electrophoresed in a 1.8 % agarose gel in 1X TBE running buffer (0.05 M Tris base, 0.05 M boric acid, 1 mM disodium EDTA), according to the methods of Preston, *et al.*, *Cancer Res.*, **1994**, *54*, 4214, incorporated herein by reference in its entirety.

Example 8: Tumorigenesis

To determine the ability of C6 cells and derivative lines to produce tumors, syngeneic BD IX rats (Charles River Breeders Laboratories, Boston, MA) were injected subcutaneously with 10^7 cells, as described in Resnicoff, *et al.*, incorporated herein by reference (*Cancer Res.*, **1994**, *54*, 2218). Wild type C6 cell sat this concentration give palpable tumors in 4 days, and the animals usually die or have to be killed after 20-25 days. Rats that did not develop tumors were kept under observation for as long as 62 days. The rats used in the diffusion chamber experiments were then used to determine the host response that is induced in rats by cells with decreased numbers of IGF-1Rs, as set forth in Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218.

Example 9: Generating Cell Lines Expressing Soluble IGF-1R

R⁻ cells were co-transfected with the pIGFIRsol and pdeltaSUpac (containing the puromycin resistance gene), and the cells were selected in 2.5 μg/ml of puromycin. Zhou-Li, *et al.*, *Mol. Cell. Biol.*, **1995**, *15*, 4232, incorporated herein by

reference. After selection and screening, several clones were obtained from three different original cell lines: Balb/c 3T3, R^- cells and C6 rat glioblastoma cells. The Balb/c 3T3 and R^- derived clones were used largely to prepare conditioned medium to be tested on other cell types. C6 cells, wild type or stably transfected with plasmid pIGFIRsol were used as such.

Several clones of C6 rat glioblastoma cells stably transfected with plasmid pIGFIRsol were selected and screened. Most of these clones grew significantly more slowly in 10% serum (not shown), and these clones were then tested for the ability to grow in SFM with or without IGF-1 (20 ng/ml). The results of a representative experiment are shown in Figure 3. C6 cells at 37°C grow quite well in SFM, even without the addition of IGF-1. Resnicoff, *et al.*, *Cancer Res.*, **1995**, *55*, 2463. Clones 5, 6 and 7 (all expressing the soluble IGF-1R having 486 amino acids) were markedly inhibited under the same conditions, roughly an 80% inhibition. These and other clones were then tested for the ability to form colonies in soft agar. Wild type C6 cells form colonies in soft agar (Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848), but, with the sole exception of clone 1, all the clones expressing the soluble receptor were markedly impaired in their anchorage independence (Table 1). Inhibition ranged from 46 to 60 percent. This is quite remarkable, because C6 cells have abundant IGF-1 receptors, and produce also some IGF-1. When wild type C6 cells are seeded with the addition of their own conditioned medium, the number of colonies more than doubles.

Table 1

Anchorage-independent Growth of C6 Rat Glioblastoma Cells Expressing Soluble IGF-1R

cell line	number of colonies in soft agar
C6	301,317
C6 plus conditioned medium from wt C6	> 1,000
C6/SR clone 1	417
clone 4	136
clone 5	167,148
clone 6	100
clone 7	129,122

C6 cells, wild type or stably transfected with plasmid pIGFIRsol, were seeded in soft agar at a density of $5x10^3$. Colonies >125 μm in diameter were scored after 2 weeks.

Example 10: Soft Agar Assay

C6 and C6/IGFIRsol cells were seeded at $5x10^3$ cells/35 mm plate in DMEM containing 10% FBS and 0.2% agarose (with 0.4% agarose underlay). C6 cells were also plated at the same density in conditioned medium from C6 or C6/IGFIRsol clones with 10% FBS and 0.2% agarose. Colonies larger than 125 μm were scored after 2 and 1 week, respectively. p6 or T/W cells were plated at the density of $1x10^3$ cells/35 mm plate in conditioned medium from R^-/IGFIRsol or Balb/IGFIRsol cells, with 10% FBS and 0.2% agarose (with 0.4% agarose underlay). Colonies larger than 125 μm in diameter were scored after 2 (p6) or 3 (T.W) weeks.

Example 11: Effects of Expression of Soluble IGF-1R

The effects of expression of the soluble IGF-1R on apoptosis of C6 cells placed in the diffusion chamber were determined next. The results are summarized in Table 2.

Table 2

C6 Rat Glioblastoma Cells Expressing Soluble IGF-1R Undergo Apoptosis In Vivo

cell line	percent recovery
wild type	215 %
clone 4	4 %
clone 6	8 %
clone 5	0.1 %
clone 7	2 %

Apoptosis was determined *in vivo* as described in Resnicoff, *et al.*, *Cancer Res.*, **1995**, *55*, 2463 and in Example 7. In each case, $5x10^5$ cells were inoculated into the diffusion chamber, which was then inserted into the subcutaneous tissue of BD IX rats. Cell numbers were determined after 24 hours and are expressed as percentage of cells originally inoculated.

As usual, wild type C6 cells grow very well in the diffusion chamber, more than doubling their number after 24 hours *in vivo*. Four clones of C6 cells expressing the soluble receptor were also tested in the diffusion chamber. All of them underwent apoptosis, the recovery of cells ranging from 0.1 to 8 percent. In short, C6 cells expressing the soluble receptor behave like C6 cells expressing an antisense RNA to the IGF-1R RNA (Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 2218; Resnicoff, *et al.*, *Cancer Res.*, **1994**, *54*, 4848; and Resnicoff, *et al.*, *Cancer Res.*, **1995**, *55*, 2463), *i.e.*, they are growth inhibited in monolayers, and in soft agar, and undergo apoptosis *in vivo*.

Example 12: C6 Cells Expressing Soluble IGF-1R Are No Longer Tumorigenic

Four clones of C6 cells expressing the soluble IGF-1R were injected subcutaneously into BD IX rats (three animals per clone) and none of them developed tumors, while control animals, injected with wild type C6 cells, promptly developed

tumors that brought about the death of the animals in 20-25 days (Table 3). The animals injected with the C6 cells expressing the soluble IGF-1R protein having 486 amino acids remained free of tumors for more than three months. To test for the immune response, the animals of Table 2 (who were implanted with diffusion chambers loaded with either wild type C6 cells or with C6 cells expressing the soluble receptor) were used. All animals were tumor-free (since the chambers had been removed to count the surviving cells), and they were challenged two weeks later with wild type C6 cells. Of the animals implanted with chambers containing the C6/SolRec cells, which express soluble IGF-1R, none developed tumors when challenged with wild type C6 cells. Rats implanted with diffusion chambers containing wild type C6 cells developed fatal tumors after subcutaneous injection with wild type C6 cells (Table 3).

Table 3

Expression of Soluble IGF-1R Abrogates Tumorigenesis

cell type injected	challenge	tumors/no. of animals
wild type	none	12/12
soluble IGF-1R	none	0/12
wild type	wild type	14/14
soluble IGF-1R	wild type	0/12

BD IX rats were injected subcutaneously with either wild type C6 cells or C6 cells expressing soluble IGF-1R. The latter animals have been kept for 90 days without the appearance of tumors. In the second half of the experiment, rats which were implanted for 24 hours with a diffusion chamber containing either wild type C6 cells or C6 cells expressing the soluble IGF-1R. Nine days after removal of the diffusion chamber, the rats were challenged with 10^7 wild type C6 cells.

The results set forth in Table 3 can be compared to results achieved by antisense therapy. Although antisense oligonucleotides inhibited tumorigenesis in nude mice, eventually all the animals developed tumors, even at the

highest concentrations of oligonucleotide (Resnicoff, *et al.*, *Cancer Res.*, **1995**, *55*, 3739). In contrast, when soluble IGF-1R was used, none of the animals developed tumors (Table 3 above and Table 3 of D'Ambrosio, *et al.*, *Cancer Res.*, **1996**, *56*, 4013). One rationale for the higher effectiveness of the soluble IGF-1R is that it is secreted into the environment and can therefore act on neighboring cells, even those that do not carry it. The uniqueness of this receptor, coupled with the lack of toxicity on normal cells, offers a higher therapeutic index (Baserga, *Trends in Biotech.*, 1996, *14*, 150, incorporated herein by reference).

Example 13: Effect of Conditioned Medium from Cells

The effect of the medium conditioned by cells expressing soluble IGF-1R on the growth of p6 cells, which are 3T3 cells overexpressing the wild type human IGF-1R, was examined. Conditioned medium from R cells stably transfected with plasmid pIGFIRsol was collected and the growth of p6 cells determined. Conditioned medium from cell lines expressing soluble IGF-1R was prepared from pIGFIRsol-transfected cells growing at 80-90% confluence. The cells were washed with Hank's Solution and incubated in serum free medium (DMEM + 0.5 mg/ml BSA + 50 mg/ml transferrin) for 72 hours. Alternatively, the serum free medium can be prepared from DMEM and 2.5 mM $FeSO_4$. Conditioned medium was collected and centrifuged at 3000 rpm for five minutes to discard dead cells. Figure 4 shows the results obtained with the conditioned medium from some of the clones. In SFM, the addition of conditioned medium has no effect on cell number, indicating that it is non toxic. However, it markedly inhibited the growth of p6 cells stimulated with IGF-1.

The conditioned medium from the same sources was then used to study its effect of colony formation in soft agar. For this purpose, T/W cells, formerly designated as (tsA)W cells (Sell, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 11217 and Sell, *et al.*, *Mol. Cell. Biol.*, **1994**, *14*, 3604), which are 3T3-like cells expressing the SV40 large T antigen, were used.

These cells form colonies in soft agar (Table 4). When conditioned medium from several clones of R⁻ cells stably transfected with plasmid pIGFIRsol, was added to the assay, it markedly inhibited colony formation, the inhibition ranging from 47 to 86 percent (Table 4).

Table 4

Effect of Conditioned Medium from R⁻ Cells Expressing Soluble IGF-1R on Soft Agar Growth of T/W Cells

treatment	# of colonies in soft agar
none	147
conditioned medium from R⁻ cells	168,159
conditioned medium from clones 1, 5, 8, 10, 14	38, 21, 78, 38, 56

T/W cells were seeded at a density of $1x10^3$ in 10% serum, without or with the conditioned medium from R⁻ cells stably transfected with plasmid pIGFIRsol. Colonies >125 μm in diameter were scored after 3 weeks.

Conditioned medium from Balb/c 3T3 cells expressing the soluble receptor was tested for its ability to inhibit colony formation in soft agar of p6 cells, that are 3T3 cells overexpressing the wild type human IGF-1 receptor. The results are summarized in Table 5. Conditioned medium from several selected clones were tested, and they inhibited soft agar colony formation of p6 cells. The inhibition ranged from 75 to 85%.

Table 5

Effect of Conditioned Medium from 3T3 Cells Expressing Soluble IGF-1R on Soft Agar Growth of p6 Cells

treatment	number of colonies in soft agar
none	382,350
conditioned medium from Balb/c 3T3 cells	378,405
conditioned medium from clones A, B, C, D	84,94,90,78
conditioned medium from clones E, G, H, I	57,68,72,90
conditioned medium from clones M, O, P	124,60,59

p6 cells were seeded at a density of $1x10^3$ cells in 10% serum without or with the conditioned medium of several Balb/c 3T3 clones stably transfected with plasmid pIGFIRsol. Colonies were scored as in Table 4.

Example 14: Host Response

Table 6 shows that cells expressing soluble IGF-1R also induce a host response that completely protects rats from a subsequent challenge with wild type C6 rat glioblastoma cells. After the chambers (for studying apoptosis) were removed, the rats were kept for a week and were then injected subcutaneously with 10^7 wild type rat glioblastoma cells. As usual, rats with chambers filled with wild type C6 cells were not protected from subsequent challenge with the same cells. These rats developed tumors that were palpable after 5 days, and lethal in 18-20 days. When the C6 cells incubated in the chamber were cells expressing soluble IGF-1R, all animals were completely protected from subsequent challenge with wild type C6 cells; not a single animal developed a tumor.

Table 6

Soluble IGF-1R Induces a Host Response in Syngeneic Rats

cell line	percent recovery	tumor development
wild type	215 %	+++
clone 4	4 %	-
clone 6	8 %	-
clone 5	0.1 %	-
clone 7	2 %	-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Renato Baserga
Mariana Resnicoff
Consuelo D'Ambrosio
Andre Ferber

(ii) TITLE OF INVENTION: Method of Inducing Resistance to Tumor Growth

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris
(B) STREET: One Liberty Place - 46th Floor
(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: WORDPERFECT 6.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: N/A
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/006,699
(B) FILING DATE: 14-NOV-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Paul K. Legaard
(B) REGISTRATION NUMBER: 38,534
(C) REFERENCE/DOCKET NUMBER: TJU-2065

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (215) 568-3100
(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1458 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Leu Lys Arg Leu Glu Asn Cys Thr Val Ile Glu Gly Tyr Leu His
            20                  25                  30

ATC CTG CTC ATC TCC AAG GCC GAG GAC TAC CGC AGC TAC CGC TTC         135
Ile Leu Leu Ile Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe
        35                  40                  45
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                50                  55                  60

GCT GGC CTC GAG AGC CTC GGA GAC CTC TTC CCC AAC CTC ACG GTC          225
Ala Gly Leu Glu Ser Leu Gly Asp Leu Phe Pro Asn Leu Thr Val
                65                  70                  75

ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC GCC CTG GTC ATC TTC          270
Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr Ala Leu Val Ile Phe
                80                  85                  90

GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC AAC CTG AGG AAC          315
Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr Asn Leu Arg Asn
                95                 100                 105

ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT GAC CTC TGT          360
Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala Asp Leu Cys
               110                 115                 120

TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG GTG TCC          405
Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala Val Ser
               125                 130                 135

AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG GAC          450
Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly Asp
               140                 145                 150

CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC          495
Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr
               155                 160                 165

ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC          540
Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg
               170                 175                 180

TGC CAG AAA ATG TGC CCA AGC ACG TGT GGG AAG CGG GCG TGC ACC          585
Cys Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr
               185                 190                 195

GAG AAC AAT GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC          630
Glu Asn Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser
               200                 205                 210

GCG CCT GAC AAC GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC          675
Ala Pro Asp Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr
               215                 220                 225

TAT GCC GGT GTC TGT GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG          720
Tyr Ala Gly Val Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg
               230                 235                 240

TTT GAG GGC TGG CGC TGT GTG GAC CGT GAC TTC TGC GCC AAC ATC          765
Phe Glu Gly Trp Arg Cys Val Asp Arg Asp Phe Cys Ala Asn Ile
               245                 250                 255

CTC AGC GCC GAG AGC AGC GAC TCC GAG GGG TTT GTG ATC CAC GAC          810
Leu Ser Ala Glu Ser Ser Asp Ser Glu Gly Phe Val Ile His Asp
               260                 265                 270

GGC GAG TGC ATG CAG GAG TGC CCC TCG GGC TTC ATC CGC AAC GGC          855
Gly Glu Cys Met Gln Glu Cys Pro Ser Gly Phe Ile Arg Asn Gly
               275                 280                 285

AGC CAG AGC ATG TAC TGC ATC CCT TGT GAA GGT CCT TGC CCG AAG          900
Ser Gln Ser Met Tyr Cys Ile Pro Cys Glu Gly Pro Cys Pro Lys
               290                 295                 300
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GTC TGT GAG GAA GAA AAG AAA ACA AAG ACC ATT GAT TCT GTT ACT          945
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                305                 310                 315

TCT GCT CAG ATG CTC CAA GGA TGC ACC ATC TTC AAG GGC AAT TTG          990
Ser Ala Gln Met Leu Gln Gly Cys Thr Ile Phe Lys Gly Asn Leu
                320                 325                 330

CTC ATT AAC ATC CGA CGG GGG AAT AAC ATT GCT TCA GAG CTG GAG         1035
Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile Ala Ser Glu Leu Glu
                335                 340                 345

AAC TTC ATG GGG CTC ATC GAG GTG GTG ACG GGC TAC GTG AAG ATC         1080
Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly Tyr Val Lys Ile
                350                 355                 360

CGC CAT TCT CAT GCC TTG GTC TCC TTG TCC TTC CTA AAA AAC CTT         1125
Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu Lys Asn Leu
                365                 370                 375

CGC CTC ATC CTA GGA GAG GAG CAG CTA GAA GGG AAT TAC TCC TTC         1170
Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr Ser Phe
                380                 385                 390

TAC GTC CTC GAC AAC CAG AAC TTG CAG CAA CTG TGG GAC TGG GAC         1215
Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp Asp
                395                 400                 405

CAC CGC AAC CTG ACC ATC AAA GCA GGG AAA ATG TAC TTT GCT TTC         1260
His Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe Ala Phe
                410                 415                 420

AAT CCC AAA TTA TGT GTT TCC GAA ATT TAC CGC ATG GAG GAA GTG         1305
Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val
                425                 430                 435

ACG GGG ACT AAA GGG CGC CAA AGC AAA GGG GAC ATA AAC ACC AGG         1350
Thr Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg
                440                 445                 450

AAC AAC GGG GAG AGA GCC TCC TGT GAA AGT GAC GTC CTG CAT TTC         1395
Asn Asn Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe
                455                 460                 465

ACC TCC ACC ACC ACG TCG AAG AAT CGC ATC ATC ATA ACC TGG CAC         1440
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                470                 475                 480

CGG CCG GTA CCG GCC CCC                                            1458
Arg Pro Val Pro Ala Pro
                485
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(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1548 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Leu Leu Phe Leu Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly
                 20                  25                  30
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Leu Lys Arg Leu Glu Asn Cys Thr Val Ile Glu Gly Tyr Leu His
                50                  55                  60

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Ile Leu Leu Ile Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe
                65                  70                  75

CCC AAG CTC ACG GTC ATT ACC GAG TAC TTG CTG CTG TTC CGA GTG          270
Pro Lys Leu Thr Val Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val
                80                  85                  90

GCT GGC CTC GAG AGC CTC GGA GAC CTC TTC CCC AAC CTC ACG GTC          315
Ala Gly Leu Glu Ser Leu Gly Asp Leu Phe Pro Asn Leu Thr Val
                95                 100                 105

ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC GCC CTG GTC ATC TTC          360
Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr Ala Leu Val Ile Phe
               110                 115                 120

GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC AAC CTG AGG AAC          405
Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr Asn Leu Arg Asn
               125                 130                 135

ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT GAC CTC TGT          450
Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala Asp Leu Cys
               140                 145                 150

TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG GTG TCC          495
Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala Val Ser
               155                 160                 165

AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG GAC          540
Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly Asp
               170                 175                 180

CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC          585
Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr
               185                 190                 195

ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC          630
Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg
               200                 205                 210

TGC CAG AAA ATG TGC CCA AGC ACG TGT GGG AAG CGG GCG TGC ACC          675
Cys Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr
               215                 220                 225

GAG AAC AAT GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC          720
Glu Asn Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser
               230                 235                 240

GCG CCT GAC AAC GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC          765
Ala Pro Asp Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr
               245                 250                 255

TAT GCC GGT GTC TGT GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG          810
Tyr Ala Gly Val Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg
               260                 265                 270

TTT GAG GGC TGG CGC TGT GTG GAC CGT GAC TTC TGC GCC AAC ATC          855
Phe Glu Gly Trp Arg Cys Val Asp Arg Asp Phe Cys Ala Asn Ile
               275                 280                 285
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CTC AGC GCC GAG AGC AGC GAC TCC GAG GGG TTT GTG ATC CAC GAC          900
Leu Ser Ala Glu Ser Ser Asp Ser Glu Gly Phe Val Ile His Asp
                290                 295                 300

GGC GAG TGC ATG CAG GAG TGC CCC TCG GGC TTC ATC CGC AAC GGC          945
Gly Glu Cys Met Gln Glu Cys Pro Ser Gly Phe Ile Arg Asn Gly
                305                 310                 315

AGC CAG AGC ATG TAC TGC ATC CCT TGT GAA GGT CCT TGC CCG AAG          990
Ser Gln Ser Met Tyr Cys Ile Pro Cys Glu Gly Pro Cys Pro Lys
                320                 325                 330

GTC TGT GAG GAA GAA AAG AAA ACA AAG ACC ATT GAT TCT GTT ACT         1035
Val Cys Glu Glu Glu Lys Lys Thr Lys Thr Ile Asp Ser Val Thr
                335                 340                 345

TCT GCT CAG ATG CTC CAA GGA TGC ACC ATC TTC AAG GGC AAT TTG         1080
Ser Ala Gln Met Leu Gln Gly Cys Thr Ile Phe Lys Gly Asn Leu
                350                 355                 360

CTC ATT AAC ATC CGA CGG GGG AAT AAC ATT GCT TCA GAG CTG GAG         1125
Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile Ala Ser Glu Leu Glu
                365                 370                 375

AAC TTC ATG GGG CTC ATC GAG GTG GTG ACG GGC TAC GTG AAG ATC         1170
Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly Tyr Val Lys Ile
                380                 385                 390

CGC CAT TCT CAT GCC TTG GTC TCC TTG TCC TTC CTA AAA AAC CTT         1215
Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu Lys Asn Leu
                395                 400                 405

CGC CTC ATC CTA GGA GAG GAG CAG CTA GAA GGG AAT TAC TCC TTC         1260
Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr Ser Phe
                410                 415                 420

TAC GTC CTC GAC AAC CAG AAC TTG CAG CAA CTG TGG GAC TGG GAC         1305
Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp Asp
                425                 430                 435

CAC CGC AAC CTG ACC ATC AAA GCA GGG AAA ATG TAC TTT GCT TTC         1350
His Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe Ala Phe
                440                 445                 450

AAT CCC AAA TTA TGT GTT TCC GAA ATT TAC CGC ATG GAG GAA GTG         1395
Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val
                455                 460                 465

ACG GGG ACT AAA GGG CGC CAA AGC AAA GGG GAC ATA AAC ACC AGG         1440
Thr Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg
                470                 475                 480

AAC AAC GGG GAG AGA GCC TCC TGT GAA AGT GAC GTC CTG CAT TTC         1485
Asn Asn Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe
                485                 490                 495

ACC TCC ACC ACC ACG TCG AAG AAT CGC ATC ATC ATA ACC TGG CAC         1530
Thr Ser Thr Thr Thr Ser Lys Asn Arg Ile Ile Ile Thr Trp His
                500                 505                 510

CGG CCG GTA CCG GCC CCC                                            1548
Arg Pro Val Pro Ala Pro
                515
```

(3) INFORMATION FOR SEQ ID NO: 3:
 (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 26

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATCCTAGA AATCTGCGGG CCAGGC 26

(3) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCAGGGGGCC GGTACCGGCC 20

What is claimed is:

1. Substantially pure soluble IGF-1R protein.

2. The protein of claim 1 wherein said protein has the amino acid sequence set forth in SEQ ID NO:1, or a fragment thereof.

3. The protein of claim 1 wherein said protein has the amino acid sequence set forth in SEQ ID NO:2, or a fragment thereof.

4. A pharmaceutical composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.

5. A pharmaceutical composition comprising the protein of claim 2 and a pharmaceutically acceptable carrier.

6. A pharmaceutical composition comprising the protein of claim 3 and a pharmaceutically acceptable carrier.

7. An isolated nucleic acid molecule that comprises a nucleic acid sequence that encodes the protein of claim 1.

8. A pharmaceutical composition comprising the nucleic acid molecule of claim 7 and a pharmaceutically acceptable carrier.

9. A recombinant expression vector comprising the nucleic acid molecule of claim 7.

10. A host cell comprising the recombinant expression vector of claim 9.

11. A method of inducing resistance to tumor growth comprising administering soluble IGF-1R to a mammal for a therapeutically effective time.

12. The method of claim 11, wherein the soluble IGF-1R is administered topically, intradermally, intravenously, intramuscularly, intraperitoneally, subcutaneously, or intraosseously.

13. The method of claim 12, wherein the soluble IGF-1R is a fragment thereof.

14. A method of inducing resistance to tumor growth comprising:

providing a tumor cell culture supplemented with soluble IGF-1R protein in a diffusion chamber; and

inserting said diffusion chamber into a mammal for a therapeutically effective time.

15. The method of claim 14 wherein said tumor cells are excised from said mammal.

16. The method of claim 14 wherein said tumor cells are selected from the group consisting of melanoma, prostate, ovary, mammary, lungs, and smooth muscle.

17. The method of claim 14 wherein said tumor cells are selected from the group consisting of autografts, allografts, and xenografts.

18. The method of claim 14 wherein said mammal is human.

19. The method of claim 14 wherein said soluble IGF-1R protein is a fragment thereof.

20. A method of inducing resistance to tumor growth comprising:

providing a culture of tumor cells in a diffusion chamber; and

inserting said diffusion chamber into a mammal for a therapeutically effective time;

wherein the tumor cells are transfected with a gene construct comprising nucleic acid molecule encoding soluble IGF-1R.

21. The method of claim 20 wherein said tumor cells are excised from said mammal.

22. The method of claim 20 wherein said tumor cells are selected from the group consisting of melanoma, prostate, ovary, mammary, lungs, and smooth muscle.

23. The method of claim 20 wherein said tumor cells are selected from the group consisting of autografts, allografts, and xenografts.

24. The method of claim 20 wherein said mammal is human.

25. The method of claim 20 wherein said soluble IGF-1R protein is a fragment thereof.

26. A diffusion chamber comprising tumor cells in culture supplemented with soluble IGF-1R.

27. A diffusion chamber comprising tumor cells transfected with a gene construct comprising nucleic acid molecule encoding soluble IGF-1R.

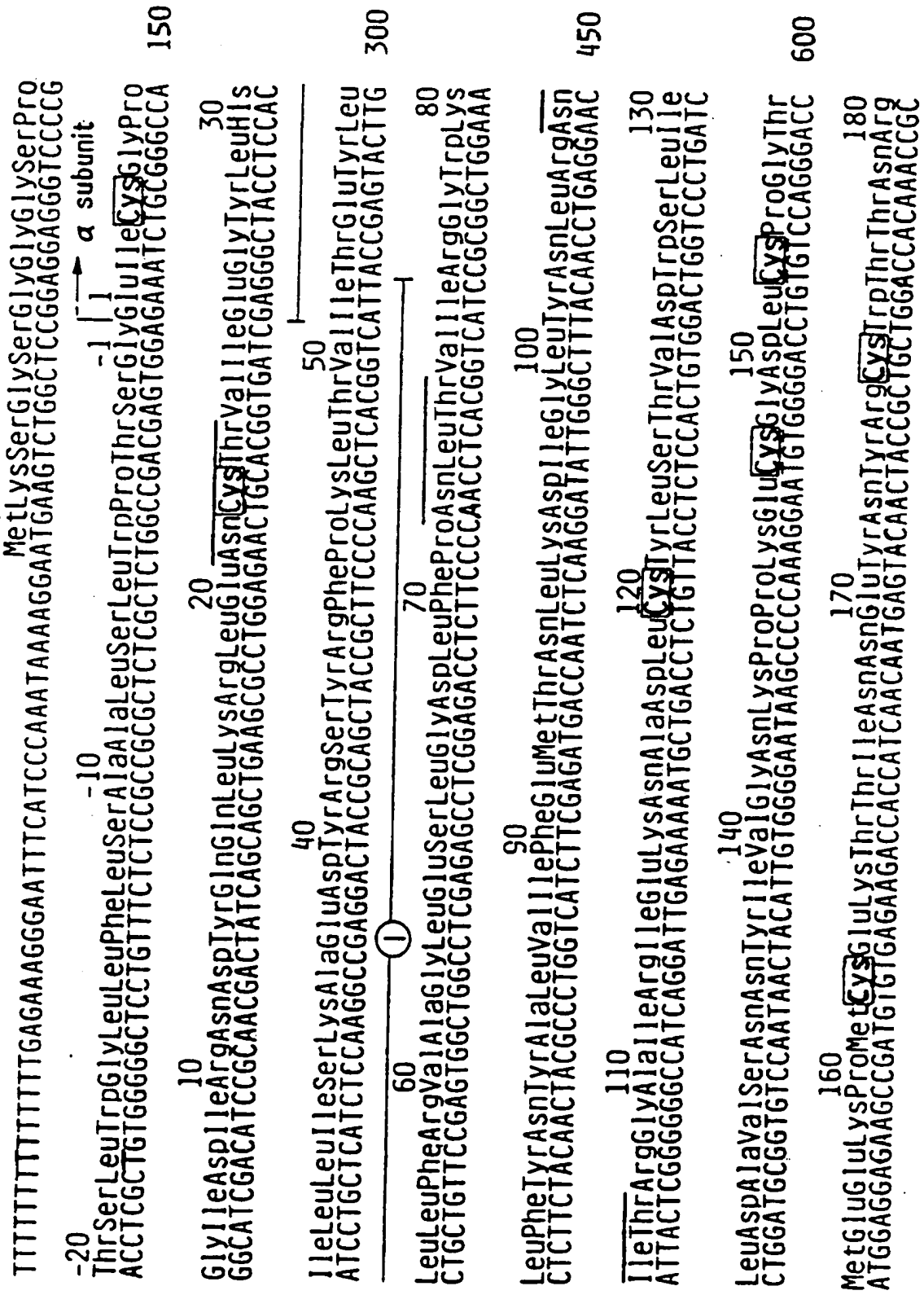

FIG. 1A

190
CysGlnLysMetCysProSerThrCysGlyLysArgAlaCysThrGluAsnAsnGluCysCysHisProGluCys 750
TGCCAGAAAATGTGCCCAAGCACGTGTGGGAAGCGGGCGTGCACCGAGAACAATGAGTGCTGCCACCCCGAGTGC
210 220 230
LeuGlySerCysSerAlaProAspAsnAspThrAlaCysValAlaCysArgHisTyrTyrAlaGlyValCys
CTGGGCAGCTGCAGCGCCTGACAACGACACGGCCTTGTGTAGCTTGCCGCCACTACTACGCCGGTGTCTGT
240 250
ValProAlaCysProProAsnThrTyrArgPheGluGlyTrpArgCysValAspArgAspPheCysAlaAsnIle 900
GTGCCTGCCTGCCCGCCCAACACCTACAGGTTTGAGGGCTGGCGCTGTGTGGACCGTGACTTCTGCGCCAACATC
260 270 280
LeuSerAlaGluSerSerAspSerGluGlyPheValIleHisAspGlyGluCysMetGlnGluCysProSerGly
CTCAGCGCCGAGAGCAGCGACTCCGAGGGGTTTGTGATCCACGACGGCGAGTGCATGCAGGAGTGCCCCTCGGGC
290 300
PheIleArgAsnGlySerGlnSerMetTyrCysIleProCysGluGlyProCysProLysValCysGluGluGlu 1050
TTCATCCGCAACGGCAGCCAGAGCATGTACTGCATCCCTTGTGAAGGTCCTTGCCCGAAGGTCTGTGAGGAAGAA
310 320 330
LysLysThrLysThrIleAspSerValThrSerAlaGlnMetLeuGlnGlyCysThrIlePheLysGlyAsnLeu
AAGAAAACAAAGACCATTGATTCTGTTACTTCTGCTCAGATGCTCCAAGGGATGCACCATCTTCAAGGGCAATTTG
340 350
LeuIleAsnIleArgArgGlyAsnAsnIleAlaSerGluLeuGluAsnPheMetGlyLeuIleGluValValThr 1200
CTCATTAACATCCGACGGGGGAATAACATTGCTTCAGAGCTGGAGAACTTCATGGGGCTCATCGAGGTGGTGACG
360 370 380
GlyTyrValLysIleArgHisSerHisAlaLeuValSerLeuSerPheLeuLysAsnLeuArgLeuIleLeuGly
GGCTACGTGAAGATCCGCCATTCTCATGCCTTGGTCTCCTTCCTTAAAAACCTTCGCCTCATCCTAGGA
390 400
GluGluGlnLeuGluGlyAsnTyrSerPheTyrValLeuAspAsnGlnAsnLeuGlnLeuTrpAspTrpAsp 1350
GAGGAGCAGCTAGAAGGGAATTACTCCTTCTACGTCCTCGACAACCAGAACTTGCAGCTGTGGGACTGGGAC

FIG. 1B

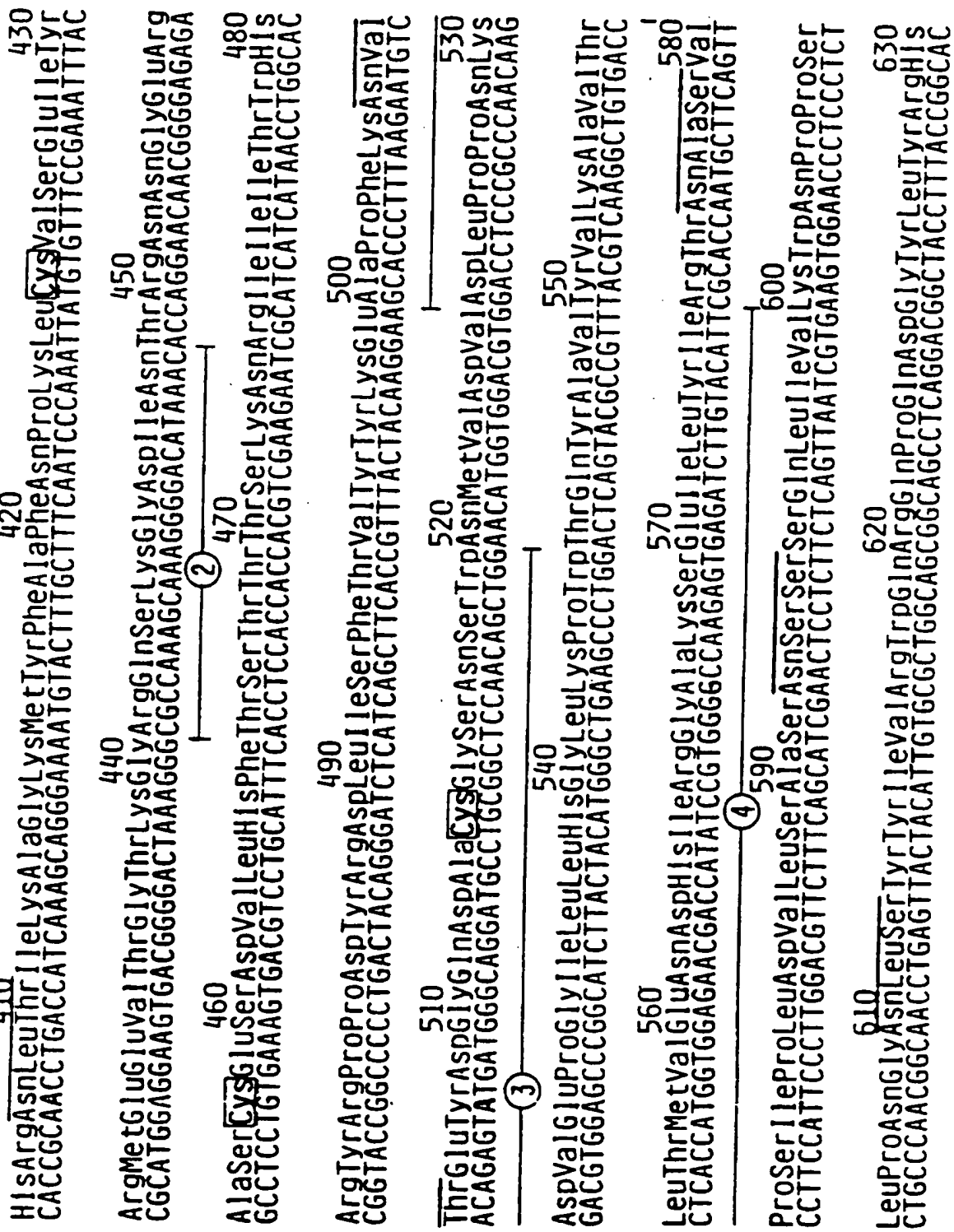

FIG. 1C

FIG. 1D

```
860                     870                      880
ArgLysTyrGlyGlyAlaLysLeuAsnArgLeuAsnProGlyAsnTyrThrAlaArgIleGlnAlaThrSerLeu
AGGAAGTATGGAGGGGCCAAGCTAAACCGGCTAAACCCGGGGAACTACACAGCCCGGATTCAGGCCACATCTCTC

890                     900
SerGlyAsnGlySerTrpThrAspProValPhePheTyrValGlnAlaLysThrGlyTyrGluAsnPheIleHis
TCTGGGAATGGGTCGTGGACAGATCCTGTGTTCTTCTATGTCCAGGCCAAAACAGGATATGAAAACTTCATCCAT 2850

910                     920                      930
LeuIleIleAlaLeuProValAlaValLeuLeuIleValGlyGlyLeuValIleMetLeuTyrValPheHisArg
CTGATCATCGCTCTGCCCGTCGCTGTCCTGTTGATCGTGGGAGGGTTGGTGATTATGCTGTACGTCTTCCATAGA

940                     950
LysArgAsnAsnSerArgLeuGlyAsnGlyValLeuTyrAlaSerValAsnProGluTyrPheSerAlaAlaAsp
AAGAGAAATAACAGCAGGCTGGGGAATGGAGTGCTGTATGCCTCTGTGAACCCGGAGTACTTCAGCGCTGCTGAT 3000

960                     970            *    *    980
ValTyrValProAspGluTrpGluValAlaArgGluLysIleThrMetSerArgGluLeuGlyGlnGlySerPhe
GTGTACGTTCCTGATGAGTGGGAGGTGGCTCGGGAGAAGATCACCATGAGCCGGGAACTTGGGCAGGGGTCGTTT

*                  990                     1000
GlyMetValTyrGluGlyValAlaLysGlyValValLysAspGluProGluThrArgValAlaIleLysThrVal
GGGATGGTCTATGAAGGAGTTGCCAAGGGTGTGGTGAAAGATGAACCTGAAACCAGAGTGGCCATTAAAACAGTG 3150

1010                    1020                     1030
AsnGluAlaAlaSerMetArgGluArgIleGluPheLeuAsnGluAlaSerValMetLysGluPheAsnCysHis
AACGAGGCCGCAAGCATGCGTGAGAGGATTGAGTTTCTCAACGAAGCTTCTGTGATGAAGGAGTTCAATTGTCAC

1040                    1050
HisValValArgLeuLeuGlyValValSerGlnGlyGlnProThrLeuValIleMetGluLeuMetThrArgGly
CATGTGGTGCGATTGCTGGGTGTGGTGTCCCAAGGCCAGCCAACACTGGTCATCATGGAACTGATGACACGGGGC 3300

1060                    1070                     1080
AspLeuLysSerTyrLeuArgSerLeuArgProGluMetGluAsnAsnProValLeuAlaProProSerLeuSer
GATCTCAAAAGTTATCTCCGGTCTCTGAGGCCAGAAATGGAGAATAATCCAGTCCTAGCACCTCCAAGCCTGAGC
```

FIG. 1E

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                                                                    1090
LysMetIleGlnMetAlaGlyGluIleAlaAspGlyMetAlaTyrLeuAsnAlaAsnLysPheValHisArgAsp  3450
AAGATGATTCAGATGGCCGGAGATTGCAGACGGCATGGCATACCTCAACGCCAATAAGTTCGTCCACAGAGAC

                                                                    1110
LeuAlaAlaArgAsnCysMetValAlaGluAspPheThrValLysIleGlyAspPheGlyMetThrArgAspIle  1130
CTTGCTGCCCGGAATTGCATGGTAGCCGAAGATTTCACAGTCAAAATCGGAGATTTTGGTATGACGCGAGATATC

                                                                    1150
TyrGluThrAspTyrTyrArgLysGlyGlyLysGlyLeuLeuProValArgTrpMetSerProGluSerLeuLys  3600
TATGAGACAGACTATTACCGGAAAGGGGGAAAGGGCTGCTGCCCGTGCGCTGGATGTCTCCTGAGTCCCTCAAG

                                                                    1170
AspGlyValPheThrThrTyrSerAspValTrpSerPheGlyValValLeuTrpGluIleAlaThrLeuAlaGlu  1180
GATGGAGTCTTCACCACTTACTCGGACGTCTGGTCCTTCGGGGTCGTCCTCTGGGAGATCGCCACACTGGCCGAG

                                                                    1190
GlnProTyrGlnGlyLeuSerAsnGluGlnValLeuArgPheValMetGluGlyGlyLeuLeuAspLysProAsp  3750
CAGCCCTACCAGGGCTTGTCCAACGAGCAAGTCCTTCGCTTCGTCATGGAGGGCGGCCTTCTGGACAAGCCAGAC

                                                                    1210
AsnCysProAspMetLeuPheGluLeuMetArgMetCysTrpGlnTyrAsnProLysMetArgProSerPheLeu  1230
AACTGTCCTGACATGCTGTTTGAACTGATGCGCATGTGCTGGCAGTATAACCCCAAGATGAGGCCTTCCTTCCTG

                                                                    1240
GluIleIleSerSerIleLysGluGluMetGluProGlyPheArgGluValSerPheTyrTyrSerGluGluAsn  3900
GAGATCATCAGCAGCATCAAAGAGGAGATGGAGCCTGGCTTCCGGGAGGTCTCCTTCTACTACAGCGAGGAGAAC

                                                                    1260
LysLeuProGluProGluGluLeuAspLeuGluProGluAsnMetGluSerValProLeuAspProSerAlaSer  1280
AAGCTGCCCGAGCCGGAGGAGCTGGACCTGGAGCCAGAGAACATGGAGAGCGTCCCCCTGGACCCCTCGGCCTCC

                                                                    1290
SerSerSerLeuProLeuProAspArgHisSerGlyHisLysAlaGluAsnGlyProGlyProGlyValLeuVal  4050
TCGTCCTCCCTGCCACTGCCCGACAGACACTCAGGACACAAGGCCGAGAACGGCCCCGGCCCTGGGGGTGCTGGTC
```

FIG. 1F

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                                                                    1310
LeuArgAlaSerPheAspGluArgGlnProTyrAlaHisMetAsnGlyGlyArgLysAsnGluArgAlaLeuPro
CTCCGCGCCAGCTTCGACGAGAGACAGCCTTACGCCCACATGAACGGGGGCCGCAAGAACGAGCGGGCCTTGCCG

                                                                    1330
LeuProGlnSerSerThrCysEnd
CTGCCCCAGTCTTCGACCTGCTGATCCTTGGATCCTGAATCTGTGCAAACAGTAACGTGTGCGCACGCGCAGCGG      4200

GGTGGGGGGGGAGAGAGAGTTTTAACAATCCATTCACAAGCCTCCTGTACCTCAGTGGATCTTCAGTTCTGCCCT      4350

TGCTGCCCGCGGGAGACAGCTTCTCTGCAGTAAAACACATTTGGGATGTTCCTTTTTTCAATATGCAAGCAGCTT      4500

TTTATTCCCTGCCCAAACCCTTAACTGACATGGGCCTTTAAGAACCTTAATGACAACACTTAATAGCAACAGAGC      4650

ACTTGAGAACCAGTCTCCTCACTCTGTCCCTGTTCTCCCTTTCTCTCCTCTCTGCTTCATAAC

GGAAAAATAATTGCCACAAGTCCAGCTGGGAAGCCCTTTTTATCAGTTTGAGGAAGTGGCTGTCCCTGTGGCCCC      4800

ATCCAACCACTGTACACACCCGCCTGACACCGTGGGTCATTACAAAAAAACACGTGGAGATGGAAATTTTTACCT

TTATCTTTCACCTTTCTAGGGACATGAAATTTACAAAGGGCCATCGTTCATCCAAGGCTGTTACCATTTTAACGC      4950

TGCCTAATTTTGCCAAAATCCTGAACTTTCTCCCTCATCGGCCCGGCGCTGATTCCTCGTGTCCGGAGGCATGGG

TGAGCATGGCAGCTGGTTGCTCCATTTGAGAGACACGCTGGCGACACACTCCGTCCATCCGACTGCCCCTGCTGT      4989

GCTGCTCAAGGCCACAGGCACACAGGTCTCATTGCTTCTGACTAGATTATTATTTGGGGGAACTGGACACAATAG

GTCTTTCTCTCAGTGAAGGTGGGGAGAAGCTGAACCGGC
```

FIG. 1G

Diffusion Chamber

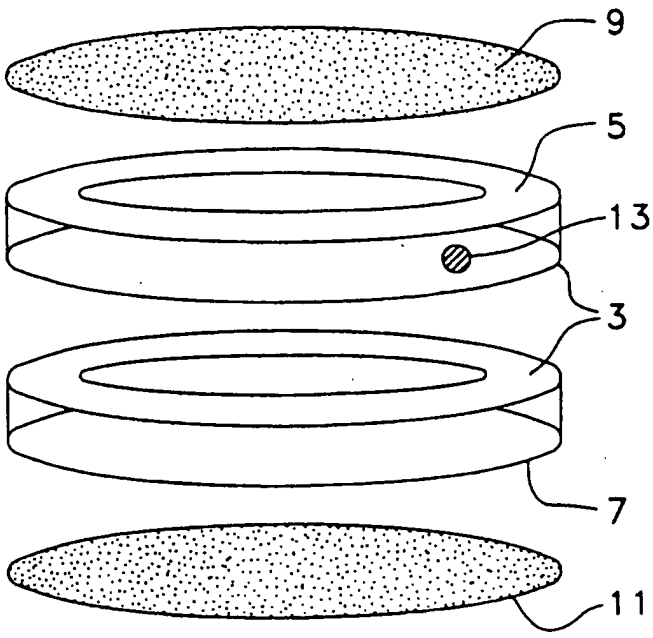

FIG. 2

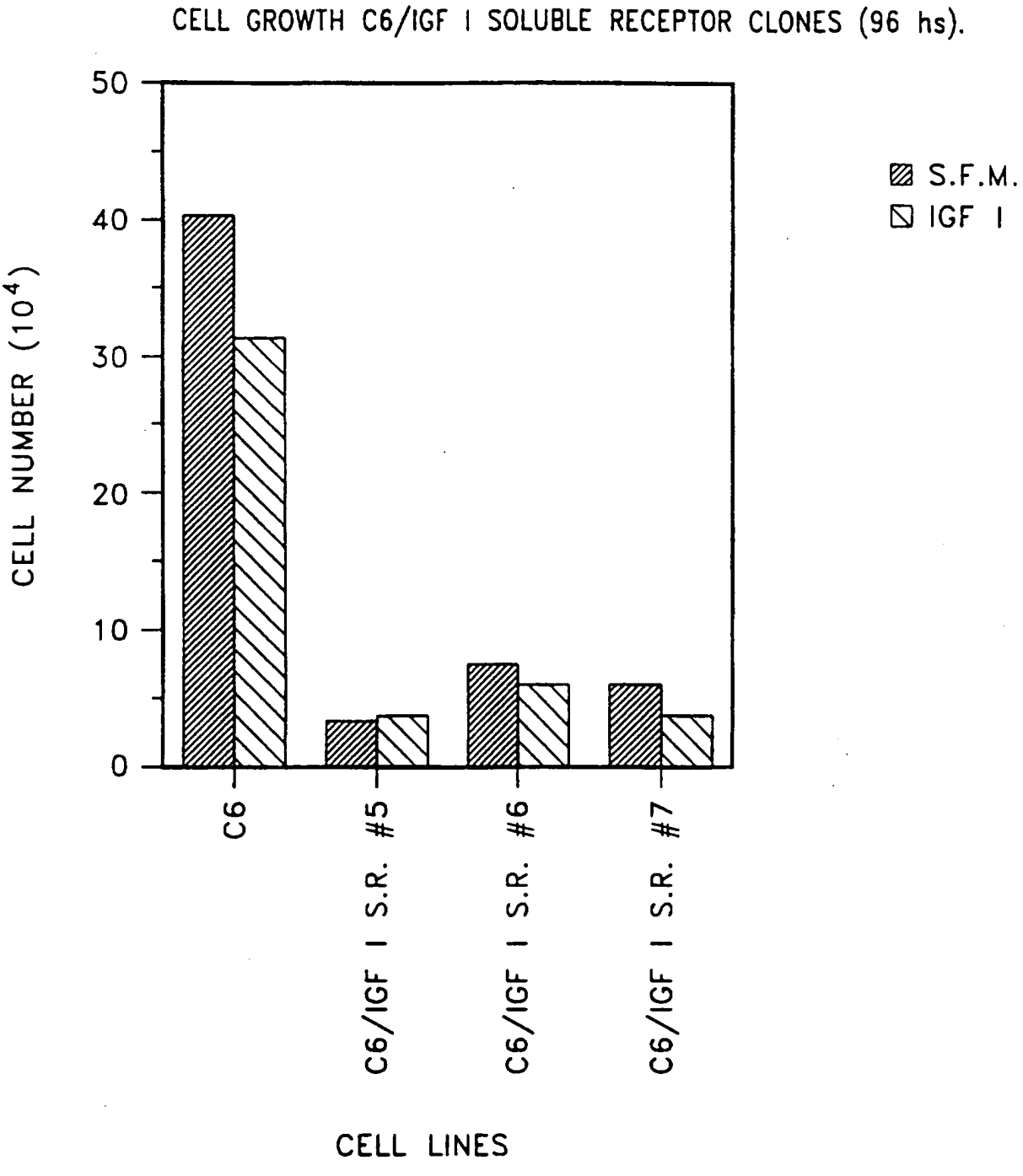
CELL GROWTH C6/IGF I SOLUBLE RECEPTOR CLONES (96 hs).
CELL NUMBER (10^4)
50
40
30
20
10
0
S.F.M.
IGF I
C6
C6/IGF I S.R. #5
C6/IGF I S.R. #6
C6/IGF I S.R. #7
CELL LINES

FIG. 3

GROWTH CURVE P6 + C.M. FROM Balb/IGF I SOLUBLE RECEPTOR CLONES w/o AND WITH IGF I (10 ng/ml). 96 HOURS.

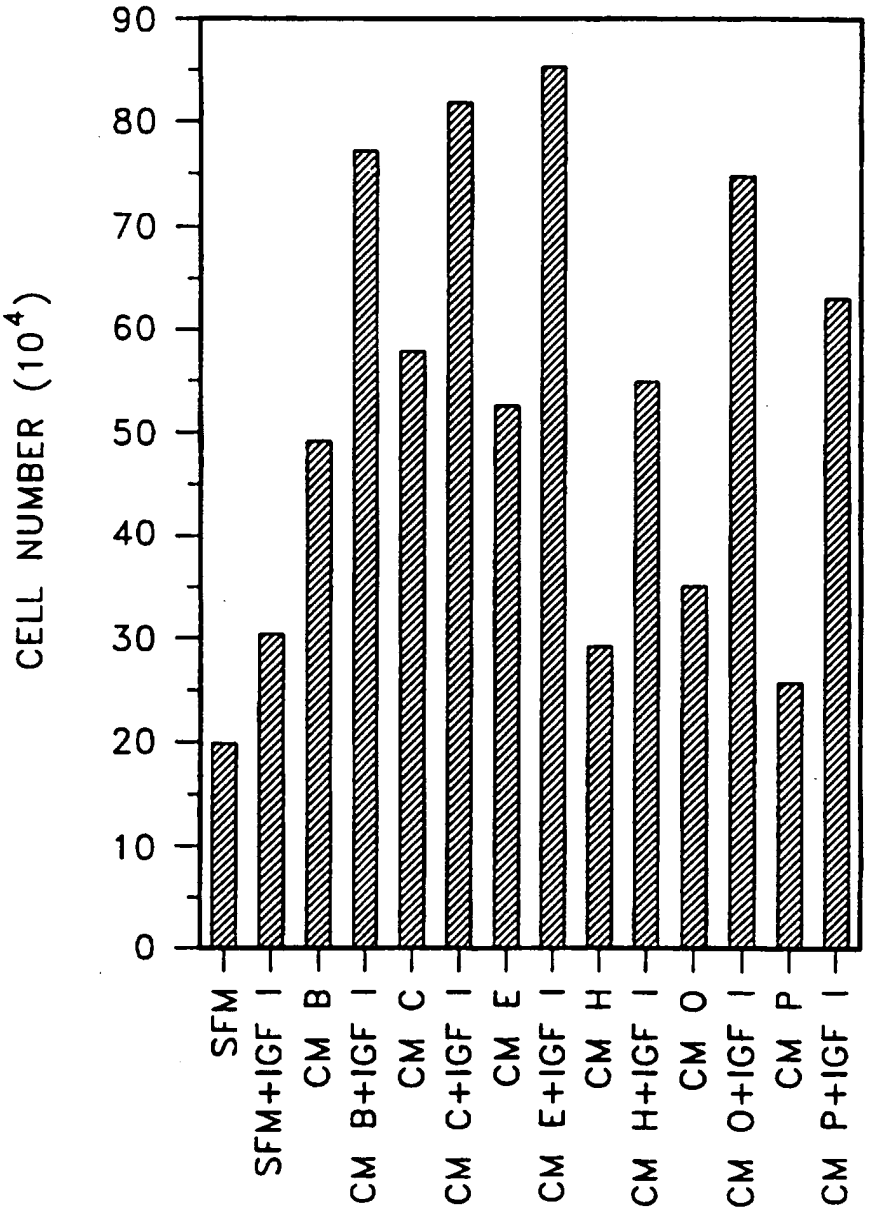

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18327

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/71; A61K 38/17, 48/00; C12N 5/10, 15/12

US CL : 530/350; 536/23.5; 435/69.1, 320.1, 336, 252.3; 514/2, 8, 44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 435/69.1, 320.1, 336, 252.3; 514/2, 8, 44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAPLUS, WPIDS, EMBL, GENBANK, GENESEQ, SWISSPRO
search terms: IGF-1, insulin-like growth factor, soluble, receptor, tumor, growth inhibition, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ULLRICH ET AL. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. The EMBO Journal. 1986, Vol.5, No.10, pages 2503-2512, see entire document, especially Figures 2 and 3.	1-10
Y	WO 91/17252 A1 (NOVO NORDISK A/S) 14 November 1991, pages 23, 26-27 and figure 4.	1-10
Y	WO 94/22486 A1(THOMAS JEFFERSON UNIVERSITY) 13 October 1994, figure 7 and pages 3-5.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 FEBRUARY 1997	21 MAR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELIANE LAZAR-WESLEY Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18327

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,262,308 (BASERGA) 16 November 1993, column 3, lines 63-68.	7-10
Y	CAMPBELL, P.G. ET AL. Insulin-like growth factor binding protein (IGFBP) inhibits IGF action on human osteosarcoma cells. Journal of Cellular Physiology. 1991. Vol. 149, pages 293-300, see abstract.	11-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18327

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-13, drawn to protein, nucleic acid, vector, host cell and method of administering protein.
Group II, claims 14-19 and 26, drawn to method of using tumor cells in combination with the protein.
Group III, claims 20-25 and 27, drawn to method of using transfected tumor cells.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The methods of groups I-III are completely different methods which are not so linked by a special technical feature. Note PCT Rule 13 does not provide for multiple methods within a single application.